SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 23 June 2021

doi: 10.2903/j.efsa.2021.6698

Safety and efficacy of a feed additive consisting of *Saccharomyces cerevisiae* MUCL 39885 (Biosprint®) for all pigs (other than sows and weaned piglets) and other minor porcine species (Prosol S.p.A.)

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Abstract

The additive Biosprint® contains viable cells of *Saccharomyces cerevisiae* MUCL 39885 and is authorised as a feed additive in sows, dairy cows, horses, piglets (weaned), cattle for fattening, minor ruminants for fattening and minor ruminants for dairy products. The applicant has requested to extend the use of the additive to all pigs (other than sows and weaned piglets) and other minor porcine species at a minimum inclusion level of 3×10^9 CFU/kg feed. *S. cerevisiae* is considered by EFSA to have qualified presumption of safety (QPS) status and consequently is considered safe for the target species, the consumers and the environment. The additive is considered as a potential skin and eye irritant and a skin and respiratory sensitiser. In previous evaluations, the FEEDAP Panel concluded that the additive is efficacious in sows and weaned piglets. In the current application, these conclusions are extrapolated to all pigs (other than sows and weaned piglets) and to other minor porcine species.

Keywords: zootechnical additive, Biosprint, *Saccharomyces cerevisiae*, renewal, QPS, pigs, other minor porcine species

Requestor: European Commission

Question number: EFSA-Q-2020-00600

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgements: The Panel wishes to acknowledge the contribution of Rosella Brozzi to this opinion.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Fašmon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Anguita M, Galobart J, Manini P, Pettenati E, Tarrés-Call J and Pizzo F, 2021. Scientific Opinion on the safety and efficacy of a feed additive consisting of *Saccharomyces cerevisiae* MUCL 39885 (Biosprint®) for all pigs (other than sows and weaned piglets) and other minor porcine species (Prosol S.p.A.). EFSA Journal 2021;19(7):6698, 9 pp. https://doi.org/10.2903/j.efsa.2021.6698

ISSN: 1831-4732

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

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1. Introduction

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

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Prosol S.p.A.[2] for authorisation of the preparation of *Saccharomyces cerevisiae* MUCL 39885 (Biosprint®), when used as a feed additive for all pigs (other than sows, suckling and weaned piglets) and other minor porcine species (category: zootechnical additives; functional group: gut flora stabiliser). During the assessment, the applicant requested a change in the species from 'for all pigs (other than sows, suckling and weaned piglets) and other minor porcine species' to 'for all pigs (other than sows and weaned piglets) and other minor porcine species.

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 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 8 January 2021.

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 environment and on the efficacy of the preparation of *S. cerevisiae* MUCL 39885 (Biosprint®) when used under the proposed conditions of use (see Section 3.1.2).

1.2. Additional information

This additive is a preparation containing viable cells of *S. cerevisiae* MUCL 39885. It is currently authorised in sows,[3] dairy cows, horses,[4] piglets (weaned),[5] cattle for fattening,[6] minor ruminants for fattening and minor ruminants for dairy products (4b1710).[7]

The EFSA FEEDAP Panel issued several opinions on the safety and efficacy of Biosprint® (*S. cerevisiae* MUCL 39885) in different target species (EFSA, 2004, 2009, 2010a,b,c; EFSA FEEDAP Panel, 2013a, 2015, 2019a,b, 2020).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[8] in support of the authorisation request for the use of Biosprint® (*Saccharomyces cerevisiae* MUCL 39885) as a feed additive.

[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] Prosol S.p.A. via Carso 99, Madone (Italy).
[3] Commission Regulation (EC) No 896/2009 of 25 September 2009 concerning the authorisation of a new use of *Saccharomyces cerevisiae* MUCL 39885 as a feed additive for sows (holder of the authorisation Prosol SpA). OJ L 256, 29.9.2009, p. 6.
[4] Commission Regulation (EU) No 1119/2010 of 2 December 2010 concerning the authorisation of *Saccharomyces cerevisiae* MUCL 39885 as a feed additive for dairy cows and horses and amending Regulation (EC) No 1520/2007 (holder of the authorisation Prosol SpA). OJ L 317, 3.12.2010, p. 9.
[5] Commission Regulation (EU) No 170/2011 of 23 February 2011 concerning the authorisation of *Saccharomyces cerevisiae* MUCL 39885 as a feed additive for piglets (weaned) and amending Regulation (EC) No 1200/2005 (holder of authorisation Prosol SpA), OJ L 49, 24.2.2011, p. 8.
[6] Commission implementing Regulation (EU) No 1059/2013 of 29 October 2013 concerning the authorisation of a preparation of *Saccharomyces cerevisiae* MUCL 39885 as a feed additive for cattle for fattening and amending Regulation (EC) No 492/2006 (holder of the authorisation Prosol SpA) OJ L 289, 31.10.2013, p. 30.
[7] Commission implementing Regulation (EU) 2016/104 of 27 January 2016 concerning the authorisation of a preparation of *Saccharomyces cerevisiae* MUCL 39885 as a feed additive for minor ruminant species for fattening and dairy production (holder of the authorisation Prosol SpA), OJ L 21, 28.1.2016, p. 71.
[8] FEED dossier reference: FAD-2020-0025.

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.[9]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Biosprint® (*Saccharomyces cerevisiae* MUCL 39885) is in line with the principles laid down in Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b).

3. Assessment

The additive contains viable cells of *S. cerevisiae* MUCL 39885. It is proposed to be used as a zootechnical additive (functional group: gut flora stabiliser) in feed for all pigs (other than sows and weaned piglets) and other minor porcine species.

The additive is currently authorised for use in sows and weaned piglets; the applicant is asking for the extension of the authorisation to all pigs and to all minor porcine species.

3.1. Characterisation

The additive contains viable cells of the strain *S. cerevisiae* MUCL 39885 (no carriers or excipients are present in the final product) and it is authorised with a minimum declared content of 1×10^9 colony forming unit (CFU)/g. The applicant proposes the same specifications as those currently authorised.

According to the applicant, no modifications have been introduced in the manufacturing process along the last nine years (EFSA FEEDAP Panel, 2010a,b,c).

The additive is marketed in two forms, 'spherical' (Biosprint® S) and 'granulated' (Biosprint® G). Compliance with the specifications set in the authorisation was confirmed by analysis of five batches of the G form (range 1.62–1.79 $\times$ 10^{10}, mean 1.70 $\times$ 10^{10} CFU/g) and five batches of the S form (range 1.69–1.77 $\times$ 10^{10}, mean 1.74 $\times$ 10^{10} CFU/g).

The same batches of the additive were analysed for microbial contamination. The results confirm compliance with limit levels set (*Escherichia coli* < 10 CFU/g, *Salmonella* spp. no detection in 25 g, filamentous fungi < 10 CFU/g, *Listeria monocytogenes* no detection in 1 g, coliforms < 100 CFU/g, *Staphylococcus aureus* < 10 CFU/g).[10]

The possible presence of chemical contaminants was measured on three recent batches of the product. In all the three batches, the concentration of heavy metals (cadmium, lead, mercury) and arsenic were below the corresponding limits of quantification (LOQs).[11] Levels of chromium (three batches below the LOQ), cobalt (two batches were below the LOQ – 0.001 mg/kg; one batch measured 0.36 mg/kg), iron (range 5.89–49.5 mg/kg), nickel (two batches were below the LOQ – 0.0013 mg/kg; one batch measured 0.09 mg/kg), copper (range 3.7–7.92 mg/kg) and zinc (range 83.5–152 mg/kg) were also investigated. In the same batches, levels of aflatoxins B1, B2, G1 and G2 were < LOQ (0.3 or 0.5 mg/kg), of deoxynivalenol < 20 μg/kg (analysed in two batches only), of ochratoxin A < 1 μg/kg (analysed in two batches only), of zearalenone < 10 μg/kg (analysed in two batches only). Levels of pesticides screened in a multiresidue analysis were < 0.01 mg/kg, with the exception of 2,4,6-trichlorophenol (0.04 mg/kg), epoxiconazole (0.076 mg/kg), tetraconazole (0.018 mg/kg) and difenoconazole (0.025 mg/kg) in one batch.[12] Based on the results, no concern is identified.

The same batches of the G form of the additive were tested in triplicate for dusting potential[13] according to the Stauber–Heubach method. The average value of dust was 260 mg/m^3 (range: 217–290 mg/m^3). The particle size distribution measured by sieving of three batches of the product for both formulations was provided. Regarding the S form, most of the particles had a diameter > 355 μm, with no

[9] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2009-0028.pdf
[10] Technical Dossier/Section II/Annex_2-3.
[11] Limits of quantification: cadmium, mercury, lead and chromium: 0.001 mg/kg, arsenic 0.005 mg/kg.
[12] Technical dossier/Section II/Annex 4-6.
[13] Technical Dossier/Section II//Annex_II_7.

particles below 125 μm. The G form of the additive had most of the particles with a diameter > 125 μm, with no particles below 90 μm.

The non-genetically modified strain of *S. cerevisiae* composing the additive is deposited in the Belgian Coordinated Collection of Microorganism Culture Collection – Mycothéque de l'Université Catholique de Louvain (BCCM/MUCL) with the accession number 39885.[14]

A phylogenetic analysis based on ribosomal and mitochondrial protein sequences deduced from whole genome sequence data confirmed the taxonomic identification of the strain as *S. cerevisiae*. The analysis of the single nucleotide polymorphisms (SNP) in the whole genome as compared the genome of *S. cerevisiae* S288C and other 6 *Saccharomyces* species was also submitted and supports the identification of the BCCM/MUCL 39885 strain as *S. cerevisiae*.[15]

3.1.1. Stability and homogeneity

Stability and homogeneity of Biosprint® has been previously assessed (EFSA FEEDAP Panel, 2010a, b,c). In the current dossier, the applicant has provided a new shelf-life study carried out in one batch of the additive stored at 25°C for 24 months, in aluminium bags under vacuum.[16] At the end of the storage period, the *S. cerevisiae* counts losses were below 0.5 log.

3.1.2. Conditions of use

The additive is intended to be used in feed for all pigs (other than sows and weaned piglets) and other minor porcine species at a minimum inclusion level of 3×10^9 CFU/kg feed.

3.2. Safety

The species *S. cerevisiae* is considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007, EFSA BIOHAZ Panel, 2020). This approach requires the identity of the strain under assessment to be conclusively established. In the context of the current application, the identity of the active agent was confirmed as *S. cerevisiae*. Accordingly, this strain is presumed safe for the target species, the consumer and the environment.

The safety for the user has been evaluated in a previous opinion (EFSA, 2010a,b,c). In 2010, the Panel concluded that the additive should be considered as a potential skin and eye irritant and a skin sensitiser and that the inhalation exposure would be minimal. No additional data were provided in the current application that would lead to a revision of the previous conclusions. Considering the proteinaceous nature of the additive, Biosprint® should be considered a respiratory sensitiser.

The applicant in support of the safety of the additive has submitted results from several literature searches.

Three literature searches on the safety of the additive for different target species, consumer, environment and user, covering the periods 2008–2018, 2018–2019 and 2019–March 2020, were evaluated by EFSA FEEDAP Panel in previous assessments (EFSA FEEDAP Panel, 2019a,b, 2020). These searches did not reveal any safety issue related to the additive under assessment.

The applicant performed two new literature searches on the safety of the additive for pigs and dogs, covering the periods 2010–June 2020 and 2010–July 2020, respectively, using the following databases: CAB Abstracts, PubMed and Scopus. It included *'Saccharomyces cerevisiae'* and other terms relevant for target species safety and for toxicological aspects. This new search retrieved 7 publications, all of them were EFSA scientific opinions regarding the use of *Saccharomyces cerevisiae* as a feed additive.

No other relevant papers were identified that would highlight a safety concern for the target species, the consumer or the environment. Moreover, no relevant papers were identified that would add any additional concerns to those already identified for the safety for the user.

[14] Technical Dossier/Supplementary_Information/Section II/Annex_II_8.
[15] Technical Dossier/Section II/Annex_9-15.
[16] Technical Dossier/Supplementary_Information/Section II/Annex_II_21.

3.2.1. Conclusions of safety

The FEEDAP Panel concludes that Biosprint® in any of its forms is considered safe for the target species, for the consumer, and the environment. The additive should be considered as a potential skin and eye irritant and a skin and respiratory sensitiser.

3.3. Efficacy

In the current application dossier, the applicant is requesting to extend the use of the additive to all pigs (other than sows and weaned piglets) and other minor porcine species.

EFSA issued two opinions concluding that the additive is efficacious in sows (at a minimum content of 6.4×10^9 CFU/kg feed) (EFSA, 2009) and in weaned piglets (at a minimum content of 4×10^9 CFU/kg feed) (EFSA, 2010c).

Considering that efficacy has been shown in weaned piglets and in sows, the Panel considers that these conclusions can be extended/extrapolated to all pigs and other minor porcine species. Therefore, the FEEDAP Panel concludes that the additive is efficacious in all growing pigs at the level of 4×10^9 CFU/kg feed, in all reproductive animals at 6.4×10^9 CFU/kg feed and in all other minor porcine species at the respective levels: growing 4×10^9 CFU/kg feed and reproductive at 6.4×10^9 CFU/kg feed.

The FEEDAP Panel noted that the applicant has requested an inclusion level lower than those indicated above (3×10^9 CFU/kg feed). However, in the absence of data, the FEEDAP Panel was not able to conclude on the efficacy of the additive when administered at the requested inclusion level.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[17] and Good Manufacturing Practice.

4. Conclusions

Biosprint® is considered safe for the target species, consumers and the environment.

The additive is considered as a potential skin and eye irritant and a skin and respiratory sensitiser.

The FEEDAP Panel concludes that the additive has the potential to be efficacious (gut flora stabiliser) in all growing pigs at the level of 4×10^9 CFU/kg feed, in all reproductive pigs at 6.4×10^9 CFU/kg feed and in all other minor porcine species at the respective levels: growing at 4×10^9 CFU/kg feed and reproductive at 6.4×10^9 CFU/kg feed.

In the absence of data, the FEEDAP Panel is not able to conclude on the efficacy of the additive when administered at the requested inclusion level (3×10^9 CFU/kg feed).

5. Documentation as provided to EFSA/Chronology

Date	Event
12/08/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
29/08/2020	Dossier received by EFSA. Biosprint® for all pigs (other than sows, suckling and weaned piglets) and other minor porcine species. Submitted by Prosol S.p.A.
07/09/2020	Reception mandate from the European Commission
08/01/2021	Application validated by EFSA – Start of the scientific assessment
08/04/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: characterisation
09/04/2021	Comments received from Member States
13/04/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
23/06/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

[17] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013a. Scientific Opinion on the efficacy of Biosprint® (*Saccharomyces cerevisiae*) as a feed additive for cattle for fattening. EFSA Journal 2013;11(4):3174, 6 pp. https://doi.org/10.2903/j.efsa.2013.3174

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Abbreviations

BCCM/MUCL	Belgian Coordinated Collection of Microorganism Culture Collection - Mycothéque de l'Université Catholique de Louvain
CFU	colony forming unit
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
MUCL	Mycothéque de l'Université Catholique de Louvain
LOQ	limit of quantification
QPS	Qualified Presumption of Safety
SNP	single nucleotide polymorphism
WGS	whole genome sequence