Safety Assessment: Outcome of assessment of *Saccharomyces cerevisiae* MUCL 39885 (Biosprint®) as a feed additive for all pigs other than sows and suckling piglets, cats and dogs, from PROSOL S.p.A.

Reference number RP24-25-26

Regulated Products Risk Assessment Unit
Science, Evidence and Research Division, FSA

Risk Assessment Team
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Regulated Product Dossier Assessment

Safety Assessment finalised: 18/08/2023

Summary

An application was submitted to the Food Standards Agency in January 2021 from PROSOL, S.p.A, Italy ("the applicant) for the authorisation of an additive (Biosprint®) containing *Saccharomyces cerevisiae* MUCL 39885 cells, under the category of 'zootechnical' additives, functional group 'gut flora stabilisers'.

The additive contains only *Saccharomyces cerevisiae* MUCL 39885 cells and is proposed to be supplied at a minimum dose of $3x10^9$ colony forming units (CFU)/kg of complete feedstuff for all pigs other than sows and suckling piglets, and a minimum of $7x10^{10}$ CFU/kg of complete feedstuff for cats and dogs. It aims to improve the faecal consistency of the target animals.

To support the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in evaluating the dossier, the Animal Feed and Feed Additives Joint Expert Group (AFFAJEG) and the Advisory Committee on Animal Feedingstuffs (ACAF) were asked to review the dossier and the supplementary information from the applicant.

A comprehensive literature review, an independent scientific evaluation and a cytotoxicity by MTT test were evaluated by AFFAJEG, and it was concluded that the additive is safe for the proposed target species, consumers and the environment. The additive should be considered a skin and respiratory sensitiser and a potential skin and eye irritant for users. AFFAJEG concluded that the additive can be considered efficacious for improving faecal consistency in weaned piglets and all *Suidae* (other than weaned piglets, suckling piglets and sows) at the proposed dose of $3x10^9$ CFU/kg of complete feed with a moisture content of 12%. The additive can be considered efficacious in all Suidae for reproduction (other than sows) at the proposed dose of $6.4x10^9$ CFU/kg of complete feed with a moisture content of 12%. The additive can be considered efficacious in dogs and cats at the proposed dose of $7x10^{10}$ CFU/kg of complete feed with a moisture content of 12%.

The views of AFFAJEG and ACAF have been taken into account in the safety assessment which represents the opinion of the FSA and FSS.

1. Introduction

The FSA and FSS have undertaken a risk assessment for a feed additive (Biosprint®, PROSOL S.p.A., Via Carso, 99, 24040, Madone (BG), Italy) containing *Saccharomyces cerevisiae* MUCL 39885, under Regulation (EC) No 1831/2003[1] under the category of 'zootechnical' additives, functional group 'gut flora stabilisers'. To support the safety assessment by FSA and FSS, the AFFAJEG and ACAF provided advice to the FSA and FSS outlined in this document.

The dossiers were evaluated on behalf of the FSA and FSS by the AFFAJEG. In line with Article 8 of 1831/2003, the assessment has considered whether the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by EFSA for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

With thanks to the members of the AFFAJEG and ACAF during the course of the assessment, who were: Professor John Wallace, Professor Nicholas Jonsson, Martin Briggs, Dr. Katrina Campbell, Professor Matthew Fisher, Susan MacDonald, Christine McAlinden, Dr. Donald Morrison, Derek Renshaw, Dr. Michael Salter, Dr. Adam Smith, Dr. Helen Warren and Dr. Nick Wheelhouse.

The three applications related to the same active substance and formulation of the product and were therefore evaluated at the same time. The dossiers were evaluated by the AFFAJEG at their April 2021 and October 2021 meetings. Further information was provided by the applicant in June 2021, responding to queries by the FSA. The conclusions by the AFFAJEG were reviewed and approved by the ACAF at their October 2022 meeting.

This document outlines the discussion and conclusions of the AFFAJEG's assessment on the safety and efficacy of *Saccharomyces cerevisiae* MUCL 39885 for the three requests of authorisation outlined below:

- a renewal of authorisation in weaned piglets;
- a new authorisation for use in all pigs and minor porcine species (other than sows and suckling and weaned piglets);
- a new authorisation for use in cats and dogs.

2. Assessment

2.1 Section II: Identity, characterisation and conditions of use

The information presented for Section II on identity, characterisation and conditions of use of the additive is common to applications RP24, RP25 and RP26.

The additive contains only *Saccharomyces cerevisiae* MUCL 39885 cells, containing a minimum of $1x10^9$ CFU/g, and an average of $15x10^9$ CFU/g, and is commercialised in two

forms, an oval, granulated form (Biosprint® G), and a spherical form (Biosprint® S). The applicant provided data from several batches supporting the specification values outlined below.

Table 1: Identity table

Active substance		
Saccharomyces cerevisiae MUCL 39885	$15x10^9$ CFU/g	
Appearance		
Micro pellets, light tan coloured having a typical yeast smell		
Chemical-physical specifications		
Dusting potential Particle size distribution	Biosprint®G – Average of 260 mg/m^3 Biosprint®S – Average of 710µm, 100% > 50µm Biosprint®G – Average between 250-355µm, distribution is equal to 99%	
Moisture (%)	< 6	
Proteins (%)	39.5-47.5	
Ashes (%)	< 8	
Microbiological profile (CFU/g)		**Method of analysis**
Alive cells count	≥$15x10^9$	EN 15789
Total count	<500000	ISO 4833
Staphylococcus aureus	<10	ISO 6888-2
Listeria monocytogenes	Abs/g	ISO 11290
Moulds	<200	ISO 21527
Coliforms	<500	ISO 9308
E. coli	<50	ISO 16649-2
Salmonella	Abs/25g	ISO 6579
Contaminants (ppm)		
Conform to UK legislation		

The microorganism *Saccharomyces cerevisiae* MUCL 39885 is a QPS (qualified presumption of safety) microorganism. The application presented a whole genome sequence (WGS) analysis and an antimicrobial resistance (AMR) set of studies, in line with guidance recommendations. The AFFAJEG evaluated the data provided and determined that the microorganism used as the active substance of the additive is identical to the QPS registered microorganism, that it is not capable of producing antimicrobial substances and therefore is free from any antibiotic activity. The microorganism does not carry antimicrobial resistance genes.

In their first evaluation, the AFFAJEG was not able to conclude on the stability of the additive in pelleted feeds. The FSA asked the applicant to provide further information on the effects of pelleting and steam on the additive's stability, safety and efficacy. The applicant provided new pelleting stability data based on a new coated form of the product. The Group evaluated the new data and concluded that the data could not be considered relevant, as differences between the coated product and the two forms described in the application dossier were too great and had wider implications for the efficacy of the additive. The applicant provided a new set of stability studies for the two

forms of the product described in the application dossier. These showed a viability of 10% (Biosprint® G) and 0.02% (Biosprint® S) when pelleting at 40°C. The AFFAJEG concluded that the high loss of viable product when pelleting or heat treating at temperatures of 40°C makes Biosprint® G and S not suitable for pelleting or heat treating.

The additive aims to supply a minimum of $3x10^9$ CFU/kg of complete feedstuff (moisture content of 12%) for all pigs other than sows and suckling piglets, and a minimum of $7x10^{10}$ CFU/kg of complete feedstuff (moisture content of 12%) for cats and dogs.

Table 2: Proposed mode of use of BIOSPRINT®.

Proposed mode of use in animal nutrition			
Additive		*Saccharomyces cerevisiae* MUCL 39885	
Registration number/EC No/No		4b 1710	
Category(-ies) of additive		Zootechnical feed additive	
Functional group(s) of additive		Gut flora stabilisers	
Description			
Composition, description		**Purity criteria**	**Method of analysis**
Preparation of *Saccharomyces cerevisiae* MUCL 39885		Containing a minimum of: $1x10^9$ CFU/g	Enumeration: pour plate method using chloramphenicol glucose yeast extract agar EN14789. Identification: polymerase chain reaction method
Trade name (if appropriate)			BIOSPRINT®
Name of the holder of authorisation (if appropriate)			--
Conditions of use			
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**
		Saccharomyces cerevisiae CFU/kg of complete feed with a moisture content of 12%	
Weaned piglets	--	$3x10^9$	--
All pigs other than sows, suckling and weaned piglets	--	$3x10^9$	--
Cats and dogs	--	$7x10^{10}$	--

2.1.1. Conclusions on Section II

The AFFAJEG concluded that the high loss of viable product when pelleting at temperatures of 40°C makes Biosprint G and S not suitable for pelleting or heat treating.

No further concerns were raised for Section II of the dossiers.

2.2 Section III: Safety

The applicant carried out a comprehensive literature review to support the evaluation of the safety of the additive.

2.2.1 Safety for the target species

The applicant identified several articles related to the effect of *Saccharomyces cerevisiae* on the different target species listed for the three applications. None of the articles described any potential negative effects for the relevant target species from exposure to the additive. The AFFAJEG reviewed the documentation provided and determined that the evidence provided, together with the QPS status of the active substance, was sufficient to conclude that the additive can be considered safe for weaned piglets, all pigs other than sows, suckling and weaned piglets, and cats and dogs.

2.2.2. Safety for the consumer

The applicant identified several articles related to the effect of *Saccharomyces cerevisiae* on the consumer. None of the articles described any potential negative effects for the consumer. The AFFAJEG reviewed the documentation provided and determined that the evidence provided, together with the QPS status of the active substance, was sufficient to conclude that the additive can be considered safe for the consumer.

2.2.3. Safety for the user

The applicant presented several sources for the evaluation of the safety for the user:

- A particle size distribution, dusting potential and exposure assessment in line with scientific guidance, concluding that no further acute inhalation toxicity studies are required due to the product's large particle size and the unlikelihood of forming inhalable dust.
- A comprehensive literature search, which did not find any articles describing negative effects by direct exposure.
- A scientific evaluation of the additive dossier and previous European Food Safety Authority (EFSA) opinions[2,3,4] by ISPESL (Istituto Superiore per la Prevenzione e la Sicurezza del Lavoro) , concluding that "*the literature does not highlight any significant aspects with regard to protecting the operator's health in relation to the possibility of damage to the respiratory system or other routes of exposure*" and recommending the use of protective gloves, clothes and respirator complying with category III of Directive 686/89/EC when handling the additive.
- A cytotoxicity by MTT test, following International Organisation for Standardisation (ISO) 10993-5, obtaining an IC50 (Half maximal inhibitory concentration) higher than 5 mg/ml on human fibroblasts, indicative of absence of significant cytotoxic potential for epithelia and mucosae.

The AFFAJEG evaluated the information presented by the applicant, as well as previous EFSA opinions on the active substance, and concluded that, due to a lack of data provided on skin and eye irritancy or skin sensitisation, the additive:

- Should be considered a potential skin and eye irritant.

- Should be considered a skin and respiratory sensitiser.

2.2.4. Safety for the environment

The applicant identified several articles related to the effect of *Saccharomyces cerevisiae* on the environment. None of the articles described any potential negative effects for the consumer. The AFFAJEG reviewed the documentation provided and determined that the evidence provided, together with the QPS status of the active substance, was sufficient to conclude that the additive can be considered safe for the environment.

2.2.5. Conclusions on safety

The AFFAJEG concluded that the additive can be considered safe for the target species, the consumer and the environment. The additive should be considered a skin and respiratory sensitiser and a potential skin and eye irritant.

2.3. Section IV: Efficacy

Efficacy for weaned piglets did not require evaluation, as it is a renewal of authorisation.

The applicant provided evidence of efficacy in previous EFSA opinions[5,6] which concluded that the additive is efficacious in piglets and sows, and claimed that these conclusions could be extrapolated to all *Suidae* other than sows, suckling and weaned piglets.

The Group evaluated the documents provided by the applicant and concluded that the additive could be considered to be efficacious in all *Suidae* (other than weaned and suckling piglets and *Suidae* for reproduction) at a dose of $3x10^9$ CFU/kg of complete feed. For all *Suidae* for reproduction purposes other than sows, the additive can be considered to be efficacious at the dose of $6.4x10^9$ CFU/kg (extrapolated from the sow efficacy trials).

The application presented three long-term efficacy trials in dogs and one in cats, following scientific guidance recommendations. Additional efficacy tables can be found in Appendix 1.

2.3.1. Studies 1, 2 and 3 in dogs

The three studies carried out in dogs aimed to evaluate the effect of the additive on the faecal consistency of dogs reared in a commercial dog breeding facility, comparing a treatment group ($1x10^9$ CFU/kg BW) to a control group (no treatment) during 36 days of experiment. The data showed that faecal dry matter increased significantly in the treated groups compared to the controls throughout the trial period. The AFFAJEG evaluated the three studies provided, concluding that they demonstrated the efficacy of the additive for increasing faecal consistency in dogs.

Table 3: Faecal dry matter (%) in long-term efficacy trials in dogs

	Study 1 in dogs			Study 2 in dogs			Study 3 in dogs		
	Ctr	**Treat**	***P-value***	**Ctr**	**Treat**	***P-value***	**Ctr**	**Treat**	***P-value***
Day 0	37.23	39.39	0.0385	34.40	36.39	0.003	38.89	42.35	0.0046
Day 7	38.16	38.71		34.43	34.83		40.63	43.85	
Day 14	35.95	38.30		33.45	40.13		39.19	40.92	
Day 21	36.91	37.41		35.91	38.69		41.63	44.66	
Day 28	38.26	38.54		36.49	42.16		39.20	42.21	
Day 35	38.14	38.79		37.10	38.14		42.03	47.02	
Overall	37.44	38.52		35.30	38.39		40.26	43.50	

2.3.2. Study 1 in cats

The study in cats aimed to aimed to evaluate the effect of the additive on the faecal consistency of cats reared in a cattery, comparing a treatment group ($7.47x10^{10}$ CFU/kg feed) to a control group (no treatment) during 36 days of experiment. The data showed that faecal dry matter increased significantly in the treated group compared to the control throughout the trial period. The AFFAJEG evaluated the study provided, concluding that it demonstrated the efficacy of the additive for increasing faecal consistency in cats.

Table 4: Faecal dry matter (%) in long-term efficacy trial in cats

	Experimental group		*P-value*		
	Control	**Treatment**	**Treatment**	**Time**	**Treatment*time**
Day 0	43.39	45.90	0.0498	0.0009	0.0277
Day 7	43.91	41.51			
Day 14	43.64	44.98			
Day 21	40.39	46.15			
Day 28	46.39	52.82			
Day 35	46.2	44.76			
Overall	43.99	46.02			

2.3.3. Conclusions on efficacy

The AFFAJEG concluded that the additive *Saccharomyces cerevisiae* MUCL 39885 (Biosprint®) can be considered to be efficacious for improving faecal consistency in:

- Weaned piglets and all *Suidae* (other than suckling piglets, sows and *Suidae* for reproduction) at the proposed dose of $3x10^9$ CFU/kg of complete feed with a moisture content of 12%.
- All *Suidae* for reproduction (other than sows) at the proposed dose of $6.4x10^9$ CFU/kg of complete feed with a moisture content of 12%.

- Dogs and cats at the proposed dose of $7x10^{10}$ CFU/kg of complete feed with a moisture content of 12%.

3. Analytical methods evaluation

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for *Saccharomyces cerevisiae* MUCL 39885[7]:

For the enumeration of the yeast probiotic strain *Saccharomyces cerevisiae* MUCL 39885 in feed additive, premixtures and feedingstuffs, the applicant proposes the ring-trial validated European Committee for Standardisation (CEN) method EN 15789:2009. This pour plate method was ring-trial validated using feed samples containing 10^9 to 10^{13} CFU *Saccharomyces cerevisiae* /kg. The CRL recommends for official control the CEN method EN 15789:2009 for the determination of the *Saccharomyces cerevisiae* MUCL 39885 in feed additive, premixtures and feedingstuffs.

Molecular methods were used by the applicant for strain identification. The CRL recommends for official control polymerase chain reaction (PCR) typing, a generally recognised standard methodology for microbial identification, for the yeast *Saccharomyces cerevisiae* strain.

FSA/FSS accept the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The data presented in the application fully identified the additive's active substance as the QPS organism *Saccharomyces cerevisiae* MUCL 39885. The additive in its two presented forms (Biosprint G, Biosprint S) is not suitable for pelleting at temperatures of 40^{o}C and above.

Data from an extensive literature review, together with the QPS status of the organism, are sufficient evidence to demonstrate the safety of the additive for the target species, consumers and the environment. The additive should be considered a skin and respiratory sensitiser and a potential skin and eye irritant for users.

Based on data from previous EFSA opinions on sows and piglets, the additive can be considered efficacious for improving faecal consistency in weaned piglets and all *Suidae* (other than weaned piglets, suckling piglets and sows) at the proposed dose of $3x10^9$ CFU/kg of complete feed with a moisture content of 12%. The additive can be considered efficacious in all Suidae for reproduction (other than sows) at the proposed dose of $6.4x10^9$ CFU/kg of complete feed with a moisture content of 12%. The additive can be considered efficacious in dogs and cats at the proposed dose of $7x10^{10}$ CFU/kg of complete feed with a moisture content of 12%.

The FSA/FSS accept the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

5. References

1. EC (European Commission), 2003. Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition. Available at https://www.legislation.gov.uk/eur/2003/1831/contents
2. EFSA (European Food Safety Authority), 2010a. Scientific Opinion on the safety and efficacy of Biosprint® (*Saccharomyces cerevisiae*) as a feed additive for dairy cows. EFSA Journal 2010;8(7):1662, 8 pp. https://doi.org/10.2903/j.efsa.2010.1662
3. EFSA (European Food Safety Authority), 2010b. Scientific Opinion on the safety and efficacy of Biosprint® (*Saccharomyces cerevisiae*) as a feed additive for horses. EFSA Journal 2010;8(7):1659, 10 pp. https://doi.org/10.2903/j.efsa.2010.1659
4. EFSA (European Food Safety Authority), 2010c. Scientific Opinion on the safety and efficacy of Biosprint® (*Saccharomyces cerevisiae*) for piglets. EFSA Journal 2010;8(10):1864, 9 pp. https://doi.org/10.2903/j.efsa.2010.1864
5. EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2019. Scientific Opinion on the assessment of the application for renewal of authorisation of Biosprint® (*Saccharomyces cerevisiae* MUCL 39885) for sows. EFSA Journal 2019;17(6):5719, 11 pp. https://doi.org/10.2903/j.efsa.2019.5719
6. EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances use in Animal Feed), 2020. Scientific Opinion on the assessment of the application for renewal of authorisation of Biosprint® (*Saccharomyces cerevisiae* MUCL 39885) as a feed additive for weaned piglets. EFSA Journal 2020;18(11):6284, 8 pp. https://doi.org/10.2903/j.efsa.2020.6284
7. EURL-FA (European Reference Laboratory for Feed Additives), 2010. Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003. *Saccharomyces cerevisiae* MUCL 39885. Available at: https://joint-research-centre.ec.europa.eu/publications/fad-2009-0028_en

6. Abbreviations

ACAF	Advisory Committee on Animal Feedingstuffs
AFFAJEG	Joint Expert Group for Animal Feed and Feed Additives
AMR	Antimicrobial resistance
CEN	European Committee for Standardisation
CFU	Colony forming unit
ISO	International Organisation for Standardisation

MUCL	Mycotheque de l’Universite catholique de Louvain
PCR	Polymerase chain reaction
QPS	Qualified presumption of safety
WGS	Whole genome sequencing

7. Appendix 1: Efficacy studies additional tables

Table 5: Body weight (kg) in long-term efficacy trials in dogs

	Study 1 in dogs			Study 2 in dogs			Study 3 in dogs		
	Ctr	**Treat**	***P-value***	**Ctr**	**Treat**	***P-value***	**Ctr**	**Treat**	***P-value***
Day 0	14.555	14.335	0.306	10.41	10.51	0.803	26.90	26.65	0.6701
Day 7	14.598	14.310		10.38	10.50		26.87	26.74	
Day 14	14.556	14.291		10.42	10.52		26.86	26.75	
Day 21	14.586	14.398		10.44	10.52		26.91	26.77	
Day 28	14.568	14.343		10.45	10.53		26.92	26.76	
Day 35	14.624	14.341		10.45	10.53		26.88	26.76	

Table 6: Feed intake (g/week) in long-term efficacy trials in dogs

	Study 1 in dogs			Study 2 in dogs			Study 3 in dogs		
	Ctr	**Treat**	***P-value***	**Ctr**	**Treat**	***P-value***	**Ctr**	**Treat**	***P-value***
0-7 days	1351	1330	0.238	1340	1396	0.203	2532	2531	0.9808
8-14 days	1344	1330		1340	1408		2532	2531	
15-21 days	1351	1330		1340	1408		2532	2531	
22-28 days	1351	1330		1340	1408		2532	2531	
29-35 days	1351	1330		1340	1408		2532	2531	

Table 7: Body weight (g) in long-term efficacy trial in cats

	Experimental group		*P-value*		
	Control	**Treatment**	**Treatment**	**Time**	**Treatment*time**
Day 0	3744	3662	0.5247	0.9998	1.000
Day 7	3759	3688			
Day 14	3769	3680			
Day 21	3786	3688			
Day 28	3800	3704			
Day 35	3820	3718			

Table 8: Feed intake (g/week/cat) in long-term efficacy trial in cats

	Experimental group		*P-value*		
	Control	**Treatment**	**Treatment**	**Time**	**Treatment*time**
1-7 days	2520	2520	1.000	1.000	1.000
8-14 days	2520	2520			
15-21 days	2520	2520			
22-28 days	2520	2520			
29-35 days	2520	2520			
Overall	2520	2520			

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