European Food Safety Authority

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SCIENTIFIC OPINION

Safety and efficacy of Biosprint® (*Saccharomyces cerevisiae*) as a feed additive for sows [1]

Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed

(Question No EFSA-Q-2008-302)

Adopted on 3 February 2009

PANEL MEMBERS

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the safety and efficacy of the product Biosprint® (*Saccharomyces cerevisiae*) as a feed additive for sows. This product is already authorised without a time limit for use in piglets, cattle for fattening and dairy cows.

Saccharomyces cerevisiae is considered by EFSA to have QPS status and therefore no assessment of safety for the target species, the consumer and the wider environment is required. The use with sows is considered unlikely to introduce hazards for users of the products not already considered. Consequently, in the present assessment the FEEDAP Panel has considered only the efficacy of the additive Biosprint® for the target species.

Evidence that the additive, when fed to sows over the entire production cycle, is able to produce a significant beneficial effect on the weight of litters and individual piglets was demonstrated in the three studies provided. Therefore, the FEEDAP Panel concludes that the efficacy of Biosprint® is demonstrated in sows at the minimum recommended dose.

Key words: zootechnical additive, micro-organism, yeast, *Saccharomyces cerevisiae*, Biosprint®, sows, piglets, efficacy, QPS

[1] For citation purposes: Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on the safety and efficacy of Biosprint® (*Saccharomyces cerevisiae*) as a feed additive for sows. *The EFSA Journal* (2009) 970, 1-9

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BACKGROUND

Regulation (EC) No 1831/2003[2] 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 the company Prosol S.p.A.[3] for authorisation of the product Biosprint®, *Saccharomyces cerevisiae* MUCL 39885, to be used as a feed additive for sows (category: zootechnical additive; functional group: other zootechnical additives) under the conditions mentioned in Table 1.

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). EFSA received directly from the applicant the technical dossier in support of this application.[4] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 18 August 2008.

The additive Biosprint® is a microbiological feed additive containing cells of the yeast *Saccharomyces cerevisiae* MUCL 39885. This product is already authorised without a time limit for use in piglets,[5] cattle for fattening[6] and dairy cows.[7]

EFSA issued one opinion on the safety of Biosprint® for dairy cows (EFSA, 2004).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety and efficacy for the target animal, user and consumer and the environment of the product Biosprint® which is a preparation of *Saccharomyces cerevisiae* (MUCL 39885), when used under the conditions described in Table 1.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group on Micro-organisms for the preparation of this opinion.

[2] OJ L 268, 18.10.2003, p.29
[3] Prosol SpA, Via Carso 99, 24040 Madone (BG), Italy
[4] Dossier reference: FAD-2008-0006
[5] OJ L 195, 27.07.2005, p.6
[6] OJ L 89, 27.03.2006, p.6
[7] OJ L 335, 19.12.2007, p.17

Table 1. **Register entry as proposed by the applicant**

Additive		*Saccharomyces cerevisiae* MUCL 39885	
Registration number/EC No/No (if appropriate)		E 1710 CE	
Category(ies) of additive		Zootechnical additive	
Functional group(s) of additive		Other zootechnical additives	
Description			
Composition, description	**Chemical formula**	**Purity criteria (if appropriate)**	**Method of analysis (if appropriate)**
Pure *Saccharomyces cerevisiae*	Preparation of *Saccharomyces cerevisiae* containing a minimum of: Powder, spherical and oval granulated forms : 1×10^{9} CFU/g additive	100% pure cell culture without carriers	Tryptone soya broth (TSB) Malt Extract Agar (MEA) Peptone salt diluition fluid (PSD) incubate 30°C 72 h. Expression:CFU/g
Trade name (if appropriate)		BIOSPRINT®	
Name of the holder of authorisation (if appropriate)		PROSOL S.p.A.	
Conditions of use			
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**	**Withdrawal period (if appropriate)**
		mg or Units of activity or CFU kg^{-1} of complete feedingstuffs		N.A.
SOWS	-	6.4×10^{9}	1.9×10^{10}	
Other provisions and additional requirements for the labelling			
Specific conditions or restrictions for use (if appropriate)		*(short description)* The additive is not stable to pelleting	
Specific conditions or restrictions for handling (if appropriate)		*(short description)* N.A.	
Post market monitoring (if appropriate)		*(short description)* N.A.	
Specific conditions for use in complementary feedingstuffs (if appropriate)		The additive is not stable to pelleting. Avoid mixing with copper and zinc.	
Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	**Species or category of animal**	**Target tissue(s) or food products**	**Maximum content in tissues**
N.A.	N.A.	N.A.	N.A.

ASSESSMENT

1. Introduction

The additive Biosprint® is a microbiological feed additive containing cells of the yeast *Saccharomyces cerevisiae* MUCL 39885. This product is already authorised without a time limit for use in piglets, cattle for fattening and dairy cows (see Background). The Company has applied for an authorisation for a period of ten years under the category of zootechnical additives (functional group: other zootechnical additives) for its use with sows.

Saccharomyces cerevisiae is considered by the European Food Safety Authority (EFSA) to have QPS status (EFSA, 2008) and therefore no assessment of safety for the target species, the consumer and the wider environment is required. In addition, the use with sows is considered unlikely to introduce hazards for users of the products not already considered. Consequently, in the present assessment the FEEDAP panel has considered only the efficacy of the additive Biosprint® for the target species.

2. Characterisation

2.1. Characterisation of the product

The active ingredient of the additive Biosprint® is the yeast *Saccharomyces cerevisiae* BCCM/MUCL 39885 (deposited in the Belgian Co-ordinated Collections of Micro-organisms / Mycothèque de l'Université Catholique de Louvain).

The product is marketed in three different forms described as: powder (particle size from 100 µ to 200 µ), granular (0.6 mm to 0.3 mm) and spherical (0.3 mm to 1.3 mm).

2.2. Conditions of use

The product is intended for use in feed for sows at a minimum content of 6.4×10^9 CFU kg^{-1} and a maximum content of 1.9×10^{10} CFU kg^{-1} feedingstuffs.

2.3. Evaluation of the analytical methods by the Community Reference Laboratory (CRL)

EFSA has verified the CRL report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the CRL report can be found in the Appendix.

3. Efficacy

Three studies involving sows from insemination to the end of the second weaning period (two cycles) were provided. They were aimed at measuring the effect of the microbial additive on the weight of the litter and individual piglets. The experiments were performed in farms located in the same European country where animals were fed with different diets, representing feeding practices used in Europe. The experimental design involved Landrace X Large White Italian type sows in a randomised block design (Control, Treatment 1 with a dose of 6.4×10^9 CFU kg^{-1}, Treatment 2 with a dose of 1.9×10^{10} CFU kg^{-1}; the doses used

were confirmed by analysis). No indication is given on the forms (powder, granular or spherical) of Biosprint® used in the trials.

The duration of trials 1,[8] 2[9] and 3[10] were 321, 313 and 296 days, respectively. The sows were housed in single pens from the first service and for the subsequent 50 days. After this period, the animals were kept in pens of three to five animals each until farrowing, and then kept in single cages. Animals were bred on slotted floors in the same room.

Statistical analysis included in the model the effect of the group, the sow and the observation time and the number of parturition applying a multivariate ANOVA for repeated measurements of SAS. Statistical analysis was applied to piglets born alive first and second farrow, piglets weaned first and second farrow, litter weight first and second farrow, litter weight at first and second weaning, individual weight at farrow, individual weight at weaning, average daily gain and mortality.

The results of those experiments are summarised in table 2. The three studies showed a significant improvement ($P < 0.01$) of individual and litter weight at weaning (day 28). The significant results seen overall were also observed in the single cycle data, where all the litters show a significant ($P < 0.01$) improvement of the weight at weaning and consequently an increase of the individual body weight. All the trials and all the farrows show a significant increase of the average daily gain (ADG). There was a numerical reduction in piglet mortality in treated groups compared to control, reaching significance at the lower dose in one trial (Trial 3).

Table 2. **Effects of Biosprint® on piglets born to treated sows (data combined from two cycles)**

		TRIAL 1			TRIAL 2			TRIAL 3		
		C	**T1**	**T2**	**C**	**T1**	**T2**	**C**	**T1**	**T2**
Sows	(n)	14	14	14	16	16	16	15	15	15
Born alive	(n)	10.29	10.43	10.29	10.77	10.69	10.77	10.47	10.58	10.48
Weaned	(n)	8.75	9.29	9.11	8.57^{a}	8.72	9.09^{b}	8.93^{a}	9.47^{b}	9.45^{b}
Litter weight at farrowing (d1)	(kg)	15.90	16.16	15.83	16.83	16.83	16.90	14.45	14.24	15.14
Litter weight at weaning (d28)	(kg)	59.55^{A}	70.92^{B}	69.23^{B}	59.98^{A}	69.43^{B}	70.26^{B}	72.13^{A}	84.28^{B}	84.65^{B}
Individual weight at farrowing (d1)	(kg)	1.54	1.55	1.54	1.56	1.58	1.58	1.38	1.35	1.45
Individual weight at weaning (d28)	(kg)	6.87^{A}	7.66^{B}	7.60^{B}	7.05^{A}	8.06^{B}	7.76^{B}	8.10^{A}	8.90^{B}	8.96^{B}
ADG	(kg/d)	0.19^{A}	0.22^{B}	0.22^{B}	0.20^{A}	0.23^{B}	0.22^{B}	0.30^{A}	0.33^{B}	0.33^{B}
Mortality rate	(%)	15	11	11	20^{a}	18^{ab}	15^{b}	15^{a}	10^{b}	10^{b}

A,B/a,b: Different superscripts indicate significant differences at A,B ($P < 0.01$) and a,b ($P < 0.05$).

[8] Technical dossier/Annexes/Report Efficacy Biosprint 1
[9] Technical dossier/Annexes/Report Efficacy Biosprint 2
[10] Technical dossier/Annexes/Report Efficacy Biosprint 3

4. Safety

The species *Saccharomyces cerevisiae* is considered by EFSA to have QPS status and not to require any specific demonstration of safety. In the view of the FEEDAP Panel, the identity of the production strain has been established. Accordingly, no further assessment of safety is required for the active agent. However, the dossier supplied by the manufacturer was prepared and submitted to EFSA before QPS was established as an assessment tool and so contains data relevant to a full safety assessment. Those have been reviewed by the FEEDAP Panel and are summarised below.

4.1. Safety for sows

A tolerance test[11] (one full cycle, 149 days) was carried out to investigate the effects of supplemented yeast (Biosprint®) at the maximum proposed dose (T1, 1.9 x 10^{10} CFU kg^{-1} of complete feedstuffs) and ten times the maximum proposed dose (T2, 1.9 x 10^{11} CFU kg^{-1} of complete feedstuffs), in gestation and lactation diets, on the performance of sows and piglets during lactation. Forty-eight Landrace x Large White Italian type F1 (Hypor) sows, homogeneous in parity, were selected for this study and divided into three groups from insemination (C**:** fed the basal diet; T1 and T2: fed the basal diet supplemented with Biosprint®). The number of piglets born alive and weaned was determined for every sow, piglets nest and individual body weight at farrowing (day 1) and at weaning (day 27) were also determined, and average daily gain was calculated. The incidence of diarrhoea and mortality was recorded in the piglets during the weaning period. Health status was daily examined by the veterinarian of the farm. Statistical analysis included in the model the effect of the group, the sow and the observation time applying a multivariate ANOVA for repeated measurements of SAS developed on nest body weight at farrowing, nest body weight at weaning, individual body weight at farrowing and individual body weight at weaning including the fixed effect of sow diet.

The mean number of piglets born alive per sow was identical among the experimental groups. The number of weaned piglets per sow was found to be significantly higher in T1 (9.69) and T2 (9.69) groups than in control animals (8.94) ($P = 0.0002$). No differences were detected in individual body or litter weight at farrowing (day 1), while higher values in both T1 and T2 were observed in individual and litter weights at weaning ($P < 0.0001$). ADG was higher in T1 and T2 animals than in the control group ($P < 0.01$), while mortality resulted in decreased rate for piglets belonging to the Biosprint® groups ($P < 0.05$). A lower incidence of diarrhoea in the piglets was detected in T1 and T2 animals than in control animals. A significant decrease of the piglet mortality rate during the trial was found in T1 and T2 animals when compared to control animals ($P < 0.05$). The positive effects observed at the maximum recommended dose were maintained after the administration of a tenfold dose of Biosprint®.

5. Post-market monitoring

No risks associated with the use of the product are foreseen. It is considered that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[12] and Good Manufacturing Practice.

[11] Technical dossier/Annexes/Report tolerance Biosprint
[12] OJ L 35, 8.2.2005, p.1

CONCLUSIONS

The FEEDAP Panel concludes that the efficacy of Biosprint® is demonstrated in sows at the recommended levels. Evidence that the additive is able to produce a significant beneficial effect on the weight of litters and individual piglets was demonstrated in the three studies provided.

Saccharomyces cerevisiae is considered by EFSA to have QPS status and therefore no assessment of safety for the target species, the consumer and the wider environment was required. The tolerance test provided by the applicant confirmed that the additive is safe for the target species.

RECOMMENDATIONS

The FEEDAP Panel recommends the following changes to the proposal for Register entry:

– The accession number MUCL 39885 be included in the description of the product

– The entry under chemical formula be amended to read "Preparation of *Saccharomyces cerevisiae* containing a minimum of 1 x 10^9 CFU/g additive in a powder, spherical or granulated form"

– The entry under purity criteria be deleted.

DOCUMENTATION PROVIDED TO EFSA

1. Biosprint®. March 2008. Submitted by Prosol SpA, Italy.
2. Evaluation report of the Community Reference Laboratory for Feed Additives on the methods(s) of analysis for Biosprint®.
3. Comments from Member States received through the ScienceNet.

REFERENCES

EFSA (European Food safety Authority), 2004. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on a request from the Commission on the safety of "Biosprint BCCM™/MUCL39885" for dairy cows. <http://www.efsa.europa.eu/EFSA/Scientific_Opinion/opinion_feedap_07_en_final1.pdf >

EFSA (European Food Safety Authority), 2008. The maintenance of the list of QPS microorganisms added to food or feed. Scientific Opinion of the Panel on Biological Hazards.

<http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/biohaz_op_ej923_qps_en.pdf?ssbinary=true>

APPENDIX

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis for Biosprint®

In the current application authorisation is sought for the microbial feed additive Biosprint® under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of Biosprint® for sows is requested. Biosprint® in one of its three commercialised forms: powder, spherical or oval granulated contains a minimum of 1 x 10^9 viable cells (c.f.u., colony-forming units) of *Saccharomyces cerevisiae* BCCM/MUCL 39885 (as active agent) per gram. The feed additive is intended to be mixed into complete feedingstuffs at a final concentration of 6.4 x 10^9 to 1.9 x 10^{10} c.f.u./kg.

For the determination of the active agent, *Saccharomyces cerevisiae* BCCM/MUCL 39885, in the feed additive, a pour plate method based on ISO 7954 and a molecular identification method (polymerase chain reaction (PCR)) are proposed by the applicant, which are considered appropriate. For the determination of the active agent *S. cerevisiae* BCCM/MUCL 39885 in premixtures and feedingstuffs the same methods are proposed by the applicant. The enumeration method was validated in a collaborative study [System. Appl. Microbiol. 2003. 26, 147-153]. The performance characteristics of the enumeration method are standard deviations for repeatability (sr) and reproducibility (sR) of around 0.17 – 0.36 $\log_{10}$ and 0.55 – 0.60 $\log_{10}$ calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively. The limits of quantification (LOQ) of this method are around 1000 colony forming units (c.f.u) per gram (g) feed additive or premixture and around 10^6 c.f.u./kg feedingstuff. These performance characteristics are considered acceptable. This method is recommended for official controls of the active agent expressed in c.f.u. in the feed additive, premixtures and feedingstuffs.

The PCR method for strain identification was ring trial validated and demonstrated a high level of correct identification between laboratories [System. Appl. Microbiol. 2004. 27, 492-500]. It is therefore considered appropriate for official controls.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.