**SCIENTIFIC OPINION**

# Scientific Opinion on the safety and efficacy of Biosprint® (*Saccharomyces cerevisiae*) as a feed additive for horses[1]

**EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)**[2,3]

European Food Safety Authority (EFSA), Parma, Italy

## 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® when used in feeds for horses at the minimum dose of 3.1 x $10^9$ and a maximum of 6.0 x $10^9$ CFU/kg of complete feedstuffs. The product is currently authorised for use in piglets, cattle for fattening, dairy cows and sows.

Biosprint® consists of viable cells of *Saccharomyces cerevisiae*, which is considered by EFSA to be suitable for the QPS approach to safety assessment. As the identity of the active agent has been established, the use of Biosprint® can be presumed safe for horses, consumers and the environment.

In the absence of data on skin and eye irritancy or skin sensitisation, Biosprint® should be considered as a potential irritant and sensitiser, and treated accordingly. Biosprint® S and Biosprint® G are unlikely to form respirable dust. Consequently, it is concluded that the inhalation exposure associated with the use of this product would be minimal.

Three digestibility studies were provided in which Biosprint® used at the minimum recommended dose consistently increased apparent fibre digestion in adult horses.

## KEY WORDS

Zootechnical additive, Biosprint®, horses, *Saccharomyces cerevisiae*, QPS, safety, efficacy

[1] On request from the European Commission, Question No EFSA-Q-2009-00753, adopted on 22 June 2010.

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## BACKGROUND

Regulation (EC) No 1831/2003[4] 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.[5] for authorisation of the product Biosprint®, *Saccharomyces cerevisiae* MUCL 39885, to be used as a feed additive for horses (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.[6] 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 11 November 2009.

The additive Biosprint® is a microbiological feed additive containing cells of the yeast *Saccharomyces cerevisiae* MUCL 39885. This product is already authorised for use in piglets,[7] cattle for fattening,[8] dairy cows[9] and sows.[10]

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the safety of Biosprint® for cattle for fattening, piglets and pigs for fattening; including the safety for the consumer, the user and the environment (EC, 1997/2003). EFSA issued one opinion on the safety of Biosprint® for dairy cows (EFSA, 2004) and one on the safety and efficacy of the same additive for sows (EFSA, 2009a).

## 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 for the target animal, consumer, user and the environment and efficacy of the product Biosprint® which is a preparation of *Saccharomyces cerevisiae* (MUCL 39885), when used under the conditions described in Table 1.

[4] OJ L 268, 18.10.2003, p. 29.
[5] Prosol SpA, Via Carso 99, 24040 Madone (BG), Italy.
[6] EFSA Dossier reference: FAD-2008-0058.
[7] OJ L 195, 27.07.2005, p. 6.
[8] OJ L 89, 27.03.2006, p. 6.
[9] OJ L 335, 19.12.2007, p. 17.
[10] OJ L 256, 29.9.2009, p. 6.

**Table 1:** Description and conditions of use of the additive as proposed by the applicant

| | |
|---|---|
| **Additive** | *Saccharomyces cerevisiae* MUCL 39885 |
| **Registration number/EC No/No** | E 1710 |
| **Category of additive** | Zootechnical additive |
| **Functional group of additive** | Gut flora stabiliser |

| **Description** | | | |
|---|---|---|---|
| Composition, description | Chemical formula | Purity criteria | Method of analysis |
| *Saccharomyces cerevisiae* | | 100% | MAPROS 19 |

| | |
|---|---|
| **Trade name** | BIOSPRINT® |
| **Name of the holder of authorisation** | PROSOL S.p.A. |

| **Conditions of use** | | | | |
|---|---|---|---|---|
| Species or category of animal | Maximum Age | Minimum content | Maximum content | Withdrawal period |
| | | CFU/kg of complete feedingstuffs | | |
| Horses | - | $3.1 \times 10^9$ | $6.0 \times 10^9$ | - |

| **Other provisions and additional requirements for the labelling** | |
|---|---|
| Specific conditions or restrictions for use | - |
| Specific conditions or restrictions for handling | - |
| Post market monitoring | - |
| Specific conditions for use in complementary feedingstuffs | - |

| **Maximum Residue Limit (MRL)** | | | |
|---|---|---|---|
| Marker residue | Species or category of animal | Target tissue(s) or food products | Maximum content in tissues |
| - | - | - | - |

## ASSESSMENT

### 1. Introduction

Biosprint® is a microbiological feed additive containing cells of the yeast *Saccharomyces cerevisiae*. This product is already authorised for use in piglets, cattle for fattening, dairy cows and sows (see Background). The applicant is now seeking the authorisation of Biosprint® under the category of zootechnical additives (functional group: gut flora stabiliser) for use in diets for horses.

*Saccharomyces cerevisiae* is considered by the European Food Safety Authority (EFSA) to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2009b). Therefore, no assessment of safety for the target species, the consumer and the wider environment is required. Consequently, in the present assessment the FEEDAP Panel has focused on the efficacy of Biosprint® when used in diets for horses.

### 2. Characterisation

#### 2.1. Characterisation of the additive

The additive is manufactured in two forms, described by the applicant as 'spherical' (Biosprint® S) and 'granulated' (Biosprint® G), with a minimum guaranteed content of viable yeasts cells of 1 x $10^9$ CFU/g. Batch to batch variation has been assessed with 35 samples of Biosprint® G showing an average of 2.1 x $10^{10}$ CFU/g, and with 13 samples for Biosprint® S with an average concentration of 1.9 x $10^{10}$ CFU/g.[11]

Biosprint® G has a mean particle size in the range of 250 to 350 µm, with no particles of a diameter smaller than 90 µm, whilst Biosprint® S has a mean particle size in the range of 250 to 710 µm, with no particles smaller than 125 µm.[12]

Critical limits for *Salmonella*, *Listeria*, *Escherichia coli* and filamentous fungi have been set. The analysis of 48 batches consistently showed levels below the limit of detection.[11] The product was also screened (three batches) for As, Cd, Hg, Pb and aflatoxins, and the results revealed levels below the detection limits.[13]

#### 2.2. Characterisation of the active agent

The production strain *Saccharomyces cerevisiae* is deposited in the Belgian Coordinated Collection of Micro-organisms – Mycotheque de l'Université de Louvain with the accession number BCCM/MUCL 39885.[14] The species identification is based on morphological and biochemical properties.[15] The identity of the strain was confirmed by the use of Pulsed Field Gel Electrophoresis and RADP.[16]

#### 2.3. Production process

Biosprint® is produced by batch fermentation with a standard yeast medium based on molasses as a carbohydrate source. After centrifugation, the yeast is washed and further centrifuged to give a paste of 30–32 % dry matter. The paste is then either dried on a continuous fluid bed to give the 'granular' preparation (G) or in a dryer at low temperature to give the 'spherical' preparation (S).

[11] Technical dossier/Section II.
[12] Technical dossier/Section II.
[13] Supplementary information/Annexes II.2, II.3, II.4.
[14] Technical dossier/Section II/Annex II.2.
[15] Technical dossier/Section II/Annexes II.3 and II.4.
[16] Technical dossier/Section II/Annexes II.5 and II.6.

### 2.4. Stability and homogeneity

The applicant demonstrated shelf life of both formulations (four batches of each) of Biosprint® (G and S) for a period of 24 months when stored at 25 ºC in an aluminium foil packaging under vacuum. Data provided at a higher temperature (40 ºC) were too poorly described to allow consideration.[17]

Very limited data have been provided to support the stability of Biosprint® in complete feed and vitamin/mineral premixtures.

One experiment investigated the stability of a single batch of Biosprint® G when added to a horse premixture for three months at 25ºC/60% RH. In a second experiment, the stability of a single batch of Biosprint® (form not specified) when added to a horse premixture for six months was tested (40ºC/75%RH). In both cases, no significant losses were seen over the study period. A third experiment provided could not be considered because no information on storage conditions were included.[18]

Two experiments tested the stability of one batch (per test) in a commercial horse feed stored for three months at 25ºC/60% RH (G form) and 40ºC/75% RH, respectively. In both cases, no significant losses were seen over the study period. In a third experiment, the stability of one batch of Biosprint® S was tested in four different mash feeds (cattle, pigs, dairy cows and horses) over three months. As the storage conditions were not specified, these data could not be considered.[19]

The ability of Biosprint® (S and G) to form homogeneous mixtures was studied in two feedingstuffs (based on distillers grains, soybean meal and guar gum meal). The coefficient of variation from the analysis of ten subsamples was found to be approximately 3 % in both cases.[20]

### 2.5. Conditions of use

The product is intended for use in complete feed for horses at a minimum content of $3.1 \times 10^9$ and a maximum content of $6.0 \times 10^9$ CFU/kg feedstuffs.

### 2.6. 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 agent in animal feed. The Executive Summary of the CRL report can be found in the Appendix.

## 3. Safety

*Saccharomyces* is considered by the EFSA to be suitable for the QPS approach to safety assessment (EFSA, 2009b). In the view of the FEEDAP Panel, the identity of the production strain is established as *Saccharomyces* and therefore no further assessment of safety for the target species, consumer and the environment is required.

### 3.1. Safety for the user

No data on skin and eye irritancy or skin sensitisation has been provided, therefore Biosprint® should be considered as a potential irritant and sensitiser, and treated accordingly.

On the basis of the particle size distribution, Biosprint® S and Biosprint® G are unlikely to form respirable dust. It is concluded that the inhalation exposure associated with the use of this product would be minimal.

[17] Technical dossier/Section II.
[18] Technical dossier/Section II and Supplementary information/Annexes II.9 and II.10.
[19] Technical dossier/Section II and Supplementary information/Annexes II.11 and II.12.
[20] Supplementary information/Annexes II.13 and II.14.

## 4. Efficacy

Three short-term balance studies were provided, all performed in the same country but in different locations. Common diets for horses were used in the experiment including hay (twice a day) and a concentrate (three times a day). Biosprint® G (2 x $10^{10}$ CFU/g) was administered as a top dressing on the concentrate (twice a day). The doses used were the minimum recommended dose: 2 g/head/day, corresponding approximately to 3 x $10^9$ CFU/kg feed, and the maximum recommended dose: 4 g/head/day, corresponding approximately to 6 x $10^9$ CFU/kg feed. Feed was provided restrictively at maintenance level (no feed refusals). Faecal samples were collected three times a day (directly from the rectum) in the first two experiments. In the third study, total faecal production was collected and a sample extracted. The endpoints measured were: dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF). In the first two trials, the acid detergent lignin (ADL) was used as an internal marker, while in the third one the acid insoluble ash (AIA) was used for the same purpose. The data were analysed using ANOVA, considering each horse as experimental unit.

*Trial 1*

The study involved 12 horses (3–18 years old) allocated to three treatments (control, Biosprint® at the minimum and at the maximum proposed doses) based on the estimated body weight.[21] Each treatment was replicated twice. The trial was divided in two phases, an adaptation phase of 40 days and a second phase in which faeces were collected for 16 days in triplicate (56 days of experimental diet, 16 of observations). During phase one, animals were maintained in single boxes overnight, while they had access to single paddocks during daytime. In the second phase, animals were maintained in single boxes all day. In both phases the horses were maintained on shavings to avoid any nutrient ingestion other than the experimental diets.

*Trial 2*

The experimental design was a 3 x 3 Latin square involving four horses (two groups of one horse, one of two horses; 5–9 years old).[22] The experiment included three treatments: control and Biosprint® at the minimum or at the maximum proposed doses. The trial was divided in three periods of three phases each, consisting of 21 days of control feed (phase 1), 14 days of treatment (phase 2) and 7 days of treatment and collection (phase 3). During phases 1 and 2, animals were maintained in single boxes overnight, while they had access to single paddocks during daytime. In the third phase, horses were maintained on shavings to avoid any nutrient ingestion other than the experimental diets.

*Trial 3*

Six mares (11–20 years old) were allocated to two groups of three subjects in a cross-over design on the basis of age and body weight.[23] The treatments were control and Biosprint® at the minimum proposed dose. The trial was divided in two consecutive 35-day periods, each consisting of three phases (14 days of control feed, 18 days of treatment and 3 days of treatment and collection of faeces). Animals were housed and fed individually during the whole trial, with the exception of a two-hour break for exercising in the first two phases. In both phases the horses were maintained on shavings to avoid any nutrient ingestion other than the experimental diets.

[21] Technical dossier/Section IV/Annex IV.2.
[22] Technical dossier/Section IV/Annex IV.4.
[23] Technical dossier/Section IV/Annex IV.6.

**Table 2:** Summary of the efficacy studies of Biosprint® with horses

| Trial | Biosprint® (CFU/kg feedstuffs) | Digestibility (%) | | | |
|---|---|---|---|---|---|
| | | Dry matter | Crude protein | Neutral detergent fibre | Acid detergent fibre |
| 1 | 0 | $58.8^{A}$ | $66.4^{A}$ | $45.6^{A}$ | $40.3^{A}$ |
| | $3.1 \times 10^9$ | $67.6^{B}$ | $72.9^{B}$ | $56.3^{B}$ | $49.8^{B}$ |
| | $6.0 \times 10^9$ | $64.6^{B}$ | $71.4^{B}$ | $56.6^{B}$ | $51.7^{B}$ |
| 2 | 0 | $41.7^{a}$ | $56.4^{a}$ | $26.1^{a}$ | 28.2 |
| | $3.1 \times 10^9$ | $40.9^{a}$ | $56.7^{a}$ | $31.8^{b}$ | 27.1 |
| | $6.0 \times 10^9$ | $51.8^{b}$ | $66.7^{b}$ | $40.2^{b}$ | 36.3 |
| 3 | 0 | $60.1^{a}$ | 77.0 | $35.9^{a}$ | $28.0^{a}$ |
| | $3.1 \times 10^9$ | $64.5^{b}$ | 79.1 | $42.5^{b}$ | $36.5^{b}$ |

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

The addition of the minimum recommended dose of Biosprint® to the feed resulted in a significant increase of digestibility of DM, CP, NDF, ADF in trial 1, an increase of digestibility of NDF in trial 2 and an increase in digestibility of DM, NDF, ADF in trial 3.

## 5. 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[24] and Good Manufacturing Practice.

## CONCLUSIONS

The active agent *Saccharomyces cerevisiae* is considered by EFSA to be suitable for the QPS approach to safety assessment. As the identity of the active agent has been established, the use of Biosprint® can be presumed safe for horses, consumers and the environment.

In the absence of data on skin and eye irritancy or skin sensitisation, Biosprint® should be considered as a potential irritant and sensitiser, and treated accordingly. Biosprint® S and Biosprint® G are unlikely to form respirable dust. Consequently, it is concluded that the inhalation exposure associated with the use of this product would be minimal.

Biosprint® at the minimum recommended dose is efficacious in horses by improving apparent fibre digestibility.

## RECOMMENDATIONS

The FEEDAP Panel makes the following recommendations:

- the minimum guaranteed concentration of the active agent in Biosprint® (CFU/g) should be included in the description of the product,
- the accession number MUCL 39885 should be included in the description of the product,
- the dose should also be expressed as CFU/head/day.

## DOCUMENTATION PROVIDED TO EFSA

1. Biosprint® BCCN/MUCL 39885. Request for authorisation of use of the microorganism. Species under extension: horses. August 2009. Submitted by Prosol SpA, Italy.

[24] OJ L 35, 8.2.2005, p. 1.

2. Supplementary information on Biosprint® BCCN/MUCL 39885 for horses. January 2010. Submitted by Prosol SpA, Italy
3. Evaluation report of the Community Reference Laboratory for Feed Additives on the methods(s) of analysis for Biosprint®.
4. Comments from Member States received through the ScienceNet.

## REFERENCES

EC (European Commission), 1997, updated 2003. Opinion on the use of certain micro-organisms as additives in feedingstuffs.

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. The EFSA Journal 26, 1-6.

EFSA (European Food safety Authority), 2009a. 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 970, 1-9.

EFSA (European Food Safety Authority), 2009b. The maintenance of the list of QPS microorganisms added to food or feed. Scientific Opinion of the Panel on Biological Hazards. EFSA Journal, 7(12):1431.

## APPENDIX

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

In the current application authorisation is sought for the microbial feed additive *Saccharomyces cerevisiae* MUCL 39885 under the category 'zootechnical additives', functional group 'gut flora stabilizers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorization is sought for the use of *Saccharomyces cerevisiae* MUCL 39885 for horses. The feed additive is intended to be mixed at a minimum dose of $3 \times 10^9$ to $6 \times 10^9$ CFU/kg of feedingstuffs.

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 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 performance characteristics of the CEN method using CGYE (yeast extract glucose chloramphenicol) agar - reported after logarithmic transformation (CFU) - are:

- a repeatability standard deviation ($s_r$) ranging from 0.17 to 0.36 $\log_{10}$ CFU/g,

- a reproducibility standard deviation ($s_R$) ranging from 0.55 to 0.60 $\log_{10}$ CFU/g, and,

- a limit of detection (LOD) of $1 \times 10^5$ CFU/kg, well below the minimum dose proposed by the applicant.

Based on these acceptable performance characteristics, 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 PCR typing, a generally recognised standard methodology for microbial identification, for the yeast *Saccharomyces cerevisiae* strain.

Further testing or validation is not considered necessary.