

ADOPTED: 4 July 2017

AMENDED: 14 September 2017

doi: 10.2903/j.efsa.2017.4944

Safety and efficacy of Levucell[®] SC (*Saccharomyces cerevisiae* CNCM I-1077) as a feed additive for dairy cows, cattle for fattening, minor ruminant species and camelids

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Guido Rychen, Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Secundino López Puente, Marta López-Alonso, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Roberto Edoardo Villa, Robert John Wallace, Pieter Wester, Rosella Brozzi and Maria Saarela

Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Levucell[®] SC for dairy cows, cattle for fattening, all minor ruminant species and camelids. The additive consists of viable cells of a strain of *Saccharomyces cerevisiae* and is sold in three formulations. The FEEDAP Panel considers that the three available formulations are equivalent when used to deliver the same dose. The active agent fulfils the requirements of the qualified presumption of safety approach to the assessment of safety and since no concerns are expected from other components, Levucell[®] SC can be presumed safe for target animals, consumers of products from treated animals and the environment. Levucell[®] SC is not a skin irritant or sensitiser but is an eye irritant. Inhalation exposure is unlikely. Encapsulation is not expected to introduce hazards for users. The FEEDAP Panel is not in the position to conclude on the efficacy of Levucell[®] SC for dairy cows, minor dairy ruminant species or dairy camelids. Levucell[®] SC has a potential to improve the performance of cattle raised for fattening when supplied at a minimum dose of 8×10^9 CFU/head and day which would approximate to 6×10^8 CFU/kg complete feed. This conclusion is extended to minor ruminant species and camelids reared for meat production at the same minimum dose of 6×10^8 CFU/kg complete feed.

© 2017 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: Levucell SC, *Saccharomyces cerevisiae* CNCM I-1077, dairy cows, cattle for fattening, minor ruminant species, camelids, QPS, efficacy

Requestor: European Commission

Question number: EFSA-Q-2014-00375 and EFSA-Q-2014-00029

Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Coconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Secundino López Puente, Marta López-Alonso, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester.

Acknowledgements: The Panel wishes to thank the members of the Working Group on Microorganisms, including Ingrid Halle, member of the previous WG on Microorganisms 2012-2015.

Amendment: This scientific opinion has been amended following the adoption of the decision of the Commission on confidentiality claims submitted by the applicant, in accordance with Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The modified sections are indicated in the text.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Coconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López Puente S, López-Alonso M, Mantovani A, Mayo B, Ramos F, Villa RE, Wallace RJ, Wester P, Brozzi R and Saarela M, 2017. Scientific Opinion on the safety and efficacy of Levucell® SC (*Saccharomyces cerevisiae* CNCM I-1077) as a feed additive for dairy cows, cattle for fattening, minor ruminant species and camelids. *EFSA Journal* 2017;15(7):4944, 16 pp. <https://doi.org/10.2903/j.efsa.2017.4944>

ISSN: 1831-4732

© 2017 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs License](https://creativecommons.org/licenses/by-nd/4.0/), which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.



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



Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference.....	4
1.2. Additional information.....	4
2. Data and methodologies.....	5
2.1. Data.....	5
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Characterisation.....	5
3.1.1. Characterisation of the active agent.....	5
3.1.2. Manufacturing process and properties of the additive.....	5
3.1.3. Stability and homogeneity.....	6
3.1.4. Conditions of use.....	7
3.2. Safety.....	7
3.2.1. Safety for the target species, consumer and environment.....	7
3.2.2. Safety for the user.....	8
3.2.2.1. Conclusions on safety for the user.....	8
3.3. Efficacy.....	8
3.3.1. Efficacy for dairy cows.....	8
3.3.2. Efficacy for minor ruminant species and camelids for milk production.....	11
3.3.3. Efficacy for cattle for fattening.....	11
3.3.4. Efficacy for minor ruminant species and camelids reared for fattening.....	13
3.4. Post-market monitoring.....	14
4. Conclusions.....	14
Documentation provided to EFSA.....	14
References.....	14
Abbreviations.....	15
Appendix A – Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Methods of Analysis for Levucell SC.....	16

1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC. In addition, 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 LALLEMAND SAS² for re-evaluation and authorisation of the product Levucell® SC (*Saccharomyces cerevisiae* CNCM I-1077), when used as a feed additive for dairy cows and cattle for fattening (category: zootechnical additive; functional group: digestibility enhancer) and for calves, buffaloes and all ruminants species for fattening (category: zootechnical additive; functional groups: digestibility enhancer and gut flora stabiliser). During the assessment the applicant introduced several amendments to the original mandates: (i) added the functional group gut flora stabiliser in the mandate for dairy cows and cattle for fattening, (ii) withdrew the request for authorisation for calves, and (iii) clarified the request for authorisation for minor species which include animals for fattening and for milk production as well as all camelids (i.e., all the camelid species of the family Camelidae).³

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 10(2) (re-evaluation of an authorised feed additive) and 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 dossiers in support of these applications. The particulars and documents in support of the applications were considered valid by EFSA as of 9 October 2014 and 23 June 2014, respectively.

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 the environment and on the efficacy of Levucell® SC (*Saccharomyces cerevisiae* CNCM I-1077), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive is a preparation of *Saccharomyces cerevisiae* (CNCM I-1077). The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the safety of this product for beef and dairy cattle, including safety for the user, the consumer and the environment (European Commission, 1997, updated 2003). The FEEDAP Panel has issued several opinions on the safety and efficacy of this product, one for dairy goats and dairy ewes (EFSA, 2006a), two for leisure horses (EFSA, 2006b, 2009) and one for lambs (EFSA, 2008a).

The additive is currently authorised for use in dairy cows and cattle for fattening,⁴ dairy goats and dairy ewes,⁵ lambs⁶ and horses.⁷

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 Lallemand SAS, 19, Rue des Briquetiers BP 59, 31702 Blagnac, France.

³ Technical dossier/Supplementary information March 2017/Annexes 1a and 1b.

⁴ Commission Regulation (EC) No 1200/2005 of 26 July 2005 concerning the permanent authorisation of certain additives in feedingstuffs and the provisional authorisation of a new use of an additive already authorised in feedingstuffs plus amendments. OJ L 195, 27.7.2005, p. 6–10.

⁵ Commission Regulation (EC) No 226/2007 of 1 March 2007 concerning the authorisation of *Saccharomyces cerevisiae* CNCM I-1077 (Levucell SC20 and Levucell SC10 ME) as a feed additive. OJ L 64, 02.03.2007, p. 26–28.

⁶ Commission Regulation (EC) No 1293/2008 of 18 December 2008 concerning the authorisation of a new use of *Saccharomyces cerevisiae* CNCM I-1077 (Levucell SC20 and Levucell SC10 ME) as a feed additive. OJ L 340, 19.12.2008, p. 38.

⁷ Commission Regulation (EC) No 910/2009 of 29 September 2009 concerning the authorisation of a new use of the preparation of *Saccharomyces cerevisiae* CNCM I-1077 as a feed additive for horses (holder of authorisation Lallemand SAS). OJ L 275, 30.9.2009, p. 7.

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, 2017). This approach requires the identity of the strain to be conclusively established.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of two technical dossiers⁸ in support to the authorisation request for the use of Levucell® SC (*Saccharomyces cerevisiae* CNCM I-1077) as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003 and the applicable EFSA guidance documents.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active agent in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁹

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Levucell® SC is in line with the principles laid down in Regulation (EC) No 429/2008¹⁰ and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008b).

3. Assessment

The additive is a preparation consisting of viable dried cells of *S. cerevisiae* (CNCM I-1077) intended for use as a zootechnical additive (digestibility enhancer and gut flora stabiliser) in feed for dairy cows, cattle for fattening and all minor ruminant species and camelids to improve zootechnical performance.

3.1. Characterisation

3.1.1. Characterisation of the active agent

The *S. cerevisiae* strain was originally isolated from grape must and is deposited at Collection Nationale de Cultures de Microorganismes (CNCM, France) with the accession number CNCM I-1077.¹¹ It has not been genetically modified.

Species identification was established based on the carbohydrate fermentation patterns and by partial sequencing of the 28S rRNA gene (domain D1/D2, about 500 base pairs).¹² Strain level identification is based on δ -polymerase chain reaction (PCR) and microsatellites PCR for 13 loci.¹³ Genetic stability was shown by PCR fingerprinting and confirmed by comparison of the δ -PCR profiles of eight samples obtained from different batches of the product.¹⁴

3.1.2. Manufacturing process and properties of the additive¹⁵

The manufacturing process of the additive is detailed in the dossier. The additive is available in three forms:

⁸ FEED dossier references: FAD-2010-0120 and FAD-2013-0054.

⁹ The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2010-0120-levucell.pdf>

¹⁰ 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.

¹¹ Technical dossiers/Section II/Annex II.2.1.

¹² Technical dossier/Section II and Supplementary information October 2016/Annex 1a.

¹³ Technical dossier/Supplementary information October 2016/Annex 1b.

¹⁴ Technical dossiers/Section II/Annex II.2.3.

¹⁵ This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

- Levucell® SC20, a fine, granulated free-flowing powder with a minimal concentration of viable yeast cells of 2×10^{10} colony forming units (CFU)/g of additive (granulated form),
- Levucell® SC10 ME and Levucell® SC Titan¹⁶ (coated or microencapsulated forms), with a minimal concentration of viable yeast cells of 1×10^{10} CFU/g of additive. These two formulations differ in appearance due to a difference in the drying process (including fatty acids).¹⁷ For practical purposes, the two forms can be considered equivalent.¹⁸

The dossiers include data confirming compliance with the minimum specifications of the additive.¹⁹ Each production batch is tested for microbiological purity and the threshold levels set as follows: total aerobic bacteria $< 10^7$ CFU/g, coliforms < 10 CFU/g, *Escherichia coli* < 10 CFU/g, wild yeasts $< 10^5$ CFU/g and the absence of *Salmonella* in 25 g. Analyses of three batches (of a non-specified form) were provided and found to be in compliance with these action limits regarding total aerobic bacteria, coliforms and *Salmonella*. No data on the presence of wild yeasts or *E. coli* were provided.²⁰ In addition, the counts of staphylococci, anaerobic sulfate-reducing bacteria and *Clostridium perfringens* were determined and found to be below the detection levels (pathogenic staphylococci < 100 CFU/g, anaerobic sulfate-reducing bacteria < 10 CFU/g and *C. perfringens* < 10 CFU/g).

Action limits are set for heavy metals (cadmium < 0.5 mg/kg, mercury < 0.1 mg/kg and lead < 5 mg/kg), arsenic (< 2 mg/kg) and mycotoxins (aflatoxins B1, B2, G1, G2 and ochratoxin A ≤ 0.005 mg/kg, zearaleone ≤ 0.1 mg/kg). Three batches of the coated form were tested for heavy metals and arsenic and three of the granulated form and three of a not specified form were tested for mycotoxins and found all to be complying with specifications.²¹ Additional three batches of SC20 and three of SC Titan were tested for mycotoxins and found to be compliant with actions limits.²² Dioxins and furans contamination was also determined for the granulated and for microencapsulated products in three batches each.²³ Values for total TEQ (sum of the total dioxins and total furans) were ≤ 0.061 ng/kg and ≤ 0.075 ng/kg for the SC20 and SC10 ME forms, respectively.

The particle size distribution tested by mechanical sieving in four batches of the SC20 formulation proved that this form has less than 0.03% (w/w) particles with a diameter $< 100 \mu\text{m}$.²⁴ Analyses using optical sieving were performed with three batches of the SC20 form, seven batches of the SC10 ME form, and six of the SC Titan form.²⁵ In the first case, approximately 0.3% (v/v) of particles had diameter $< 90 \mu\text{m}$ and 0.1% (v/v) of particles $< 45 \mu\text{m}$. In the second case, the mean particle size was approximately $660 \mu\text{m}$ while the smallest particles had a diameter of $140 \mu\text{m}$. In the third case, the mean particle size was approximately $993 \mu\text{m}$ and no particles had a diameter below $50 \mu\text{m}$. The dusting potential of two batches of the SC20 form tested using the Stauber–Heubach dustometer gave a mean value of 23 mg/m^3 .²⁶

3.1.3. Stability and homogeneity

Shelf life of Levucell® SC10 ME (two batches) was assessed at three storage temperatures (40°C, 30°C and 20°C) and compared against one batch of the non-coated form, Levucell® SC20.²⁷ These were packed under nitrogen in bags made of the same material as the commercial packaging. Both forms were stable throughout the whole test periods (3 months at 40°C, for 4 months at 30°C and 12 months at 20°C).

Stability at 20°C for 24 months was tested with three batches of the SC20 form contained in sealed (under vacuum) packaging and with five batches in packaging materials in the presence of air. No losses of viability were observed when the additive was stored under vacuum. In the presence of air, minimal losses of viability were seen (decline from 3×10^{10} to 2×10^{10} CFU/g).²⁶ When stored at 5°C, two batches stored under vacuum and four batches stored under residual air showed minimal losses of viability (decline from 3×10^{10} to 2×10^{10} CFU/g).

¹⁶ This is apparently also marketed with the tradename Levucell® SC10 ME Titan.

¹⁷ Technical dossiers/Section II/Annex II.1.4a.

¹⁸ Technical dossiers/Section II/Annexes II.3.3 and II.3.4.

¹⁹ Technical dossier/Section II/Annexes II.3.3 and II.3.4.

²⁰ Technical dossiers/Section II/Annex II_1_3.

²¹ Technical dossiers/Section II and Supplementary information October 2016/Annex II_1_3.

²² Technical dossiers/Supplementary information October 2016/Annexes 2b and 2c.

²³ Technical dossier/Section II/Annex II_1_4.

²⁴ Technical dossiers/Section II/Annex II_1_5.

²⁵ Technical dossiers/Section II/Annexes II_1_6a and 6b and Supplementary information October 2016/Annexes 3c, 3d and 3e.

²⁶ Technical dossiers/Section II/Annex II_4_15 and Supplementary information October 2016/Annex 3a.

²⁷ Technical dossiers/Section II/Annex II_4_1.

Three batches of SC Titan in the original packaging were stored at 5 and 25°C (60% relative humidity (RH)) for 36 months. All batches were either stable or showed minimal losses of viability, remaining above the specifications over this period.²⁸

Two batches of SC10 ME and one of SC20 were packed under nitrogen and stored at 40°C for 3 months, at 30°C for 4 months and at 20°C for 12 months.²⁹ All batches were either stable or showed minimal losses of viability, remaining above the specifications over this period.

Further studies were done with one batch of SC20 when exposed to light at different intensities, or stored at different temperatures (4°C, 20°C, 40°C and 65°C) or moisture (RH 20%, 50%, 70–80% and at ambient room conditions) for 1 month. The product was stable at different moisture conditions, but did not tolerate storage at 65°C (complete loss of viability occurred within 24 h).³⁰

Stability of nine batches of SC20 and three and six of the coated forms was tested when mixed with a vitamin/mineral premixture for ovines, a mineral premixture for bovines and a mineral premixture for bovines and goats (none containing choline chloride).³¹ The additive was incorporated at 0.3–1% and the samples were stored in aluminium bags at 20°C for 6 months (expected levels 10^{10} – 10^{11} CFU/kg). The viability loss during this time was small (< 0.5 log) in all cases.

Stability of three batches of SC20 was tested when mixed with a mash feed for ruminants (expected concentration of 1×10^9 CFU/kg), contained in aluminium bags and stored at 20°C for 3 months.³⁰ The viability loss during this time was negligible. In the same study, three batches of the SC10 ME and three batches of the SC Titan forms were mixed with a feed for dairy ruminants (expected concentration of 5×10^9 – 1×10^{10} CFU/kg), subject to pelleting (at 50–65°C), packed in aluminium bags and stored at 20°C for 3 months.³⁰ Yeast counts were measured before and after pelleting to allow testing for survival during processing. No losses were observed in any of the counts after pelleting and after the 3 months of storage, denoting stability of both forms to pelleting and storage. In an additional study, stability of six batches of SC Titan to different pelleting conditions was tested.³² In this case, the additive was incorporated in a cow feed (at 1 g/kg) and the mixture was subject to pelleting at 70, 75 and 80°C. Differences in viable counts measured before and after pelleting at 70 and 75°C were in all batches < 0.5 log, denoting good stability to pelleting up to 75°C. Viability losses at 80 °C were slightly over 0.5 log values.

The capacity of one batch of SC20 and SC10 ME, and two of the SC Titan form to homogeneously mix with mash and pelleted feed for dairy and fattening ruminants was tested in another study.³³ The additive was added to the feed to deliver the yeast at 10^8 – 10^9 CFU/kg in the feed for dairy ruminants and at $1 \times 10^{10-11}$ CFU/kg in the feed for fattening bovines. Yeasts analyses of 10 subsamples showed coefficients of variation < 2% in all cases, denoting capacity to homogeneously mix.

3.1.4. Conditions of use

The additive is intended for use in complete feed for dairy cows and minor ruminant species for milk production at the minimum dose of 4×10^8 CFU/kg, for cattle for fattening and minor ruminant species for fattening at the minimum dose of 5×10^8 CFU/kg and for all camelids at the minimum dose of 4×10^8 CFU/kg.

3.2. Safety

3.2.1. Safety for the target species, consumer and environment

In the view of the FEEDAP Panel, the identity of the strain has been established as *S. cerevisiae* (CNCM I-1077). According to the QPS approach to safety assessment, it can be presumed safe for target animals, consumers of products derived from animals fed the additive and the environment. No concerns are expected from other components of the additive. Consequently, Levucell® SC can be presumed safe for target animals, consumers of products derived from animals fed the additive and the environment.

²⁸ Technical dossier FAD-2013-0054/Section II/Annex II_4_2b.

²⁹ Technical dossiers/Section II/Annex II_4_2.

³⁰ Technical dossiers/Section II/Annex II_4_3.

³¹ Technical dossiers/Supplementary information October 2016/Annex 4a.

³² Technical dossiers/Supplementary information October 2016/Annex 4b.

³³ Technical dossiers/Supplementary information October 2016/Annex 4c.

3.2.2. Safety for the user

No specific studies on inhalation toxicity were submitted. Although the active agent, owing to its proteinaceous nature, is considered to be a potential respiratory sensitiser, none of the formulations contains particles of respirable size. Thus, the exposure of users is unlikely. In addition, the dusting potential of the SC20 form, which is expected to be the dustiest form, is low.

Studies with five New Zealand White rabbits were made to investigate possible irritancy potential of Levucell® SC20 to skin³⁴ and eyes³⁵ following OECD guidelines 404 and 405, respectively. No skin irritation or oedema was observed after 24 and 72 h of a single application of the test material. Thus, the additive is considered non-irritant to skin. There was evidence of eye irritation with the additive, including redness, watering and chemosis at the conjunctival level in all animals. These effects lasted for 72 h. At 96 h, three of the animals showed a light redness which was not visible at 120 h. Thus, Levucell® SC20 is an eye irritant.

In an experiment compliant with OECD Guideline 429, the skin sensitisation potential of Levucell® SC20 was tested.³⁶ The test material was considered to be non-sensitiser under the test conditions.

In all of the studies described, the test item was the non-coated form. Encapsulation is not expected to introduce other hazards for users.

3.2.2.1. Conclusions on safety for the user

Levucell® SC20 is not a skin irritant or sensitiser but is an eye irritant. Inhalation exposure is unlikely. Encapsulation is not expected to introduce other hazards for users.

3.3. Efficacy

3.3.1. Efficacy for dairy cows

Eight studies performed in five different Member States were submitted to show the effects of Levucell on the performance of dairy cows. Three³⁷ studies could not be further considered due to flaws in the experimental design, as feed intake and ingestion of the additive were not determined in each cow. In addition, in one of these studies,³⁸ a poor quality silage was given during 2 weeks and caused an unacceptable reduction in feed intake and milk production.

The remaining five trials followed a similar design, which is described in Table 1. Although different forms of the additive were used in the studies, the FEEDAP Panel considers that the results of efficacy studies can be applied to any of the three available formulations when used to deliver the same dose. In all cases the intended dose is expressed in CFU/cow per day (1×10^{10} CFU/cow per day). Based on the analysed concentrations in the batch of additive used and in the premixture to which the additive was incorporated and the feed intake in each case, the equivalent dietary concentration in CFU/kg feed was calculated.

³⁴ Technical dossiers/Section III/Annex_III_3_1.

³⁵ Technical dossiers/Section III/Annex_III_3_2.

³⁶ Technical dossiers/Supplementary information October 2016/Annex 5.

³⁷ Technical dossier FAD-2010-0120/Section IV/Annexes IV_1_1, IV_1_2 and IV_1_5.

³⁸ Technical dossier FAD-2010-0120/Section IV/Annex IV_1_5.

Table 1: Details on the study design for the studies with dairy cows

Trial	Breed	Total animals (cows/treatment)	Levucell® SC in supplemented group (CFU/cow per day)	Duration of the study (days)	Diets – main ingredients (percentage of forage and concentrate in the daily ration)
1	Holstein	44 (22)	1 g Levucell® SC10 ME (1×10^{10})	104	Basal forage diet (grass and corn silage) supplemented with pelleted concentrate (60% forage, 40% concentrate)
2	Red Holstein Friesian × Simmental	36 (18)	0.5 g Levucell® SC20 (1×10^{10})	84	Partial mixed ration (maize silage, lucerne hay and protein concentrate) supplemented with concentrate (60% forage, 40% concentrate)
3	Polish Holstein-Friesian	24 (12)	1 g Levucell® SC10 ME (1×10^{10})	84	Maize silage, lucerne silage, rapeseed meal, beet pulp (60% forage, 40% concentrate)
4	Prim'Holstein	38 (19)	1 g Levucell® SC Titan (1×10^{10})	90	Partial mixed ration (maize silage, lucerne hay and protein concentrate) supplemented with concentrate (78% forage, 22% concentrate)
5	Polish Holstein-Friesian	22 (11)	1 g Levucell® SC Titan (1×10^{10})	98	Maize silage, lucerne silage, high moisture maize grain silage, rapeseed meal, beet pulp (52% forage, 48% concentrate)

CFU: colony forming unit.

In study 1, 44 Holstein cows (1/3 primiparous and 2/3 multiparous) were randomly divided into a control group and a group receiving Levucell® SC administered through concentrate in order to supply 1×10^{10} yeast cells/cow daily. Before the allocation to the groups, cows were paired according to calving date, lactation number, and previous lactation yield.³⁹ The study started when cows were on average at day 43 of lactation and lasted for 84 days. From the microbiological analysis of the concentrate (1.6×10^9 CFU/kg concentrate), it was calculated that treated cows received the additive at 6×10^8 CFU/kg complete feed. Cows were individually housed in cubicles with a central feeder where a forage based diet was provided *ad libitum*. Group forage intake was determined monthly. Cows were given a concentrate according to their milk yield up to a maximum daily amount of 10.5 kg/cow. Pelleted concentrate was available at computerised out-milking parlour individual feeders, and 1 kg of concentrate (containing the additive) was fed during milking. Daily concentrate intake was on average 5.9 and 6.5 kg/cow in the control and Levucell® SC groups, respectively. Milk yield was recorded daily for each cow, and then records of four consecutive days were averaged. Milk composition was determined every 4 weeks. Individual body condition score (BCS, 5-point scale) was assessed every 5 weeks. Milk yield data with the cow as experimental unit were analysed using a linear mixed model with the dietary treatment and parity as fixed factors, with milk recordings within each cow during the study as repeated measurements in time. Means of body condition score were compared using a Student t-test. Differences were considered as significant for $p < 0.05$.

In the second trial, 36 Red Holstein Friesian × Simmental cows were randomly distributed in two homogeneous groups (of 1 primiparous and 17 multiparous each) according to their performance in the previous lactation (milk yield and composition), body weight, BCS and the time of calving.⁴⁰ The

³⁹ Technical dossier FAD-2010-0120/Section IV/Annex IV_1_3, Supplementary information October 2016/Annex 8 and Supplementary information March 2017/Annexes 3.

⁴⁰ Technical dossier FAD-2010-0120/Section IV/Annex IV_1_4.

experiment lasted 12 weeks. The experiment started 3 weeks before calving and was extended until 12 weeks post-partum. Animals were housed in a loose stable equipped with individual feed troughs. Cows had free access to a partially mixed ration in an outside feeding area, and those producing more than 22 kg milk/day received up to 8 kg of additional concentrate, according to their performance. Cows in the treated group received the additive mixed with 1.5 kg of the concentrate. The analysed yeast concentration in the concentrate was 6.8×10^9 CFU/kg, corresponding to a calculated concentration of 4.8×10^8 CFU/kg complete feed. Those in the control group received only the concentrate without the additive. Concentrate intake was measured daily for each cow. Milk yield was automatically recorded for each cow and at each milking (twice a day). Live weight was recorded weekly, using scales installed at the exit of the milking room. All parameters were analysed by analysis of variance (ANOVA) with treatment and blocking effects included in the model with the cow as experimental unit. Means were separated using the Student–Newman–Keuls test when F-values were significant ($p < 0.10$).

In study 3, 24 Polish Holstein-Friesian multiparous (third parity) cows were blocked according to their expected calving date, and then randomly assigned to one of two treatments (control and Levucell).⁴¹ All animals entered the experiment 2 weeks before calving and were monitored during 14 weeks. In the treated group, Levucell® SC was provided in a mineral premixture that was added on top of the total mixed ration (TMR) before mixing. Animals in the control group received the same amount of mineral premixture. From the microbiological analysis of the premixture (5.2×10^{11} CFU/kg), it was calculated that treated cows received the additive at 4×10^8 CFU/kg complete feed. During the experimental period, cows from each group were kept in separate loose housing and were individually fed (Calan Broadbend gates). The TMR was fed *ad libitum*, feed consumption was measured on a daily basis and cows were milked three times. Feed intake data were computed on a weekly basis for statistical analysis. Milk production was recorded daily on an individual basis. Milk composition was determined once per month and per cow over the 12 weeks of production. Individual BCS (5-point scale) was assessed every 2 weeks. Data with the cow as experimental unit were analysed by ANOVA using a mixed model with repeated measurements. Differences were considered as significant for $p < 0.05$.

In the fourth study, 38 Prim'Holstein cows were randomly distributed in two groups.⁴² Primiparous heifers and multiparous cows were evenly distributed in both groups. Cows were paired according to the lactation number, number of days after calving, and milk production and composition at grouping. The animals were housed in a loose barn with cubicles, and a partial mixed ration was freely available at the feeding trough (one per experimental group). In addition, each cow received 1 kg of concentrate with (cows of the Levucell® SC group) or without (control group) the additive, and another kg of concentrate for every 2.5 kg of milk produced above a daily yield of 25 kg milk. Concentrates were distributed during milking. The analysed yeast cell counts in the concentrate were 8.9×10^9 CFU/kg, from which the calculated counting in complete feed was 2.8×10^8 CFU/kg. Individual concentrate consumption was recorded daily. Partial mixed ration intakes were measured on a group basis, with an assessment of total amount consumed monthly. Milk production was automatically recorded at each milking (average daily number of milkings per cow was 2.5), and milk composition was determined in milk samples taken every 2 weeks. Body condition score was determined three times along the course of the study. Data with the cow as experimental unit were subjected to ANOVA with repeated measurements. Number of days post-partum and parity at grouping were used as covariates and removed from the model when non-significant. Average milk production of the first month was used as covariate for the subsequent 13 weeks of milk production recordings.

The last study involved 22 Polish Holstein-Friesian cows blocked according to their parity, and then randomly assigned to one of two treatments (control and Levucell).⁴³ Cows entered the experiment 30 days after calving and were monitored during 14 weeks. In the treated group, Levucell® SC was provided in a premixture (with corn meal as carrier) that was added on top of the TMR before mixing. From the microbiological analysis of the premixture (2.6×10^{10} CFU/kg), it was calculated that treated cows received the additive at 2.1×10^8 CFU/kg complete feed. During the experimental period, cows from each group were kept in separate loose housing and were individually fed (Calan Broadbend gates). The TMR was fed *ad libitum*, feed consumption was measured on a daily basis and cows were milked three times per day on 8 h intervals. Milk production and feed intake were recorded daily for each cow. Milk composition was determined biweekly and per cow. Individual BCS (5-point scale) was

⁴¹ Technical dossiers/Supplementary information October 2016/Annexes 6 and Supplementary information March 2017/Annexes 2a and 2b.

⁴² Technical dossiers/Supplementary information October 2016/Annexes 7.

⁴³ Technical dossiers/Supplementary information March 2017/Annexes 2.

assessed every 2 weeks. Data with the cow as experimental unit were subjected to ANOVA using a mixed model with repeated measurements. Average milk production of the first 2 weeks was used as a covariate. Differences were considered as significant for $p < 0.05$. A summary of the results of the studies is presented in Table 2.

Table 2: Effect of Levucell® SC on milk production and milk quality

Study	Levucell® SC (CFU/kg feed)	Milk yield (kg/day)	Total feed intake (kg DM/cow per day)*	Fat content (%)	Protein content (%)	Body condition score
1	0	31.3 ^a	–	3.8	3.2	3.1
	6×10^8	33.6 ^b	–	3.9	3.2	2.9
2	0	36.2	–	3.4	3.2	nd
	5×10^8	37.2	–	3.5	3.2	nd
3	0	41.2	23.9	3.7	3.1	3.0
	4×10^8	43.0	24.0	3.7	3.0	3.0
4	0	29.2 ^a	–	4.0	3.1	2.8
	3×10^8	30.1 ^b	–	4.1	3.2	2.9
5	0	38.7 ^a	27.5	4.2	3.2	3.0 ^b
	2×10^8	42.0 ^b	27.7	4.1	3.3	2.9 ^a

CFU: colony-forming unit; DM: dry matter; nd: not determined.

a,b: Means within a column with different superscript letters are significantly different at $p < 0.05$ in all studies except in study 2 at $p < 0.1$.

*Estimated on the basis of the average group intake for forage in trials 1 (control: 15.7 kg, Levucell® SC: 16.6 kg), 2 (control: 17.3 kg, Levucell® SC: 18.5 kg) and 4 (control: 27.5 kg, Levucell® SC: 27.8 kg).

Although the results suggest that daily milk yield was significantly increased ($p < 0.10$) in three studies (1, 4 and 5), the Panel has reservations: the supply of concentrate (kg/kg milk) was based on individual milk yield (in trials 1, 2 and 4) which makes it not possible to distinguish between response to the additive or to the cumulative increases in the provision of concentrate.

The data on milk yield and feed intake of the five studies were tested for homogeneity and used in a pooled analysis.⁴⁴ However, the same reservations described above apply to this analysis.

In addition, the applicant made reference to a report published by de Ondarza et al. (2010) which described a meta-analysis of 14 research trials involving the additive under assessment and conducted in eight different countries (two of them non EU member states).⁴⁵ However, the majority of the 14 studies considered would not meet the criteria of an efficacy study, in particular in terms of duration. Some of the data used are from studies reported only in the form of abstracts.

Therefore, the FEEDAP Panel is not in the position to conclude on the efficacy of Levucell® SC for dairy cows.

3.3.2. Efficacy for minor ruminant species and camelids for milk production

An efficacy study with dairy buffaloes was provided.⁴⁶ However, this could not be further considered due to flaws in experimental design, as feed intake and ingestion of the additive were not determined in each buffalo.

In the absence of demonstration of efficacy in dairy cows, no extrapolation to minor dairy ruminant species or dairy camelids can be made.

3.3.3. Efficacy for cattle for fattening

Eight studies were submitted investigating the effects of supplementing Levucell® SC to cattle for fattening on growth performance. Four of the studies could not be further considered due to flaws in the experimental design: two^{47,48} were of too short duration (< 168 days), another one⁴⁹ lacked of

⁴⁴ Technical dossiers/Supplementary information March 2017/Annex 2i.

⁴⁵ Technical dossiers/Supplementary information March 2017/Annex 2j.

⁴⁶ Technical dossier FAD-2013-0054/Section IV/Annexes IV_1_7a-c.

⁴⁷ Technical dossier FAD-2010-0120/Section IV/Annex IV_2_1.

⁴⁸ Technical dossiers/Supplementary information October 2016/Annexes 9.

⁴⁹ Technical dossier FAD-2010-0120/Section IV/Annex IV_2_3.

replications (a single group for each treatment), and the other one⁵⁰ was discarded due to insufficient duration (149 days) and unclear description of experimental units (group feeding).

All the studies involved a comparison between a control group (no additive) and a group given a target dose of Levucell of $7\text{--}8 \times 10^8$ CFU/head per day (Table 3). Based on the analysed counts in feed or the premixture to which Levucell was added and the total feed intake, the equivalent dietary concentration in CFU/kg feed was calculated. In study 1, the additive was Levucell® SC10 ME, in study 2 it was Levucell® SC20, and in studies 3 and 4 Levucell® SC Titan was used. The FEEDAP Panel considers that the results of efficacy studies can be applied to any of the three available formulations when used to deliver the same dose.

Table 3: Details on the study design for the trials performed in cattle for fattening

Study	Breed (sex)	Total animals pen/treatment × animals/pen	Levucell® SC CFU/head per day	Duration of the study (days)	Basal diets (main ingredients)
1	Charolais ♂	96 6 × 8	Levucell® SC10 ME 8×10^9	262	Maize silage, dried sugar beet pulp, wheat, straw, soya bean meal
2	Simmental ♀	197 ¹ 6 × 16/17	Levucell® SC20 7×10^9	189	Whole crop maize, sunflower meal, soybean meal, alfalfa hay, high moisture shelled maize
3	Angus ♂	39 ² 19/20 × 1	Levucell® SC Titan 8×10^9	193	Pelleted concentrate with maize meal, corn DDG's, wheat middling, soybean, wheat, palm oil and meal
4	Charolais ♂	171 ³ 6 × 14/15	Levucell® SC Titan 7×10^9	185 (179–189)	Maize silage, high moisture ear maize, maize meal, maize gluten feed, dried sugar beet pulp, wheat straw, concentrate

CFU: colony forming unit.

1: 96 in the control and 101 in the Levucell® SC group.

2: 19 in the control and 20 in the Levucell® SC group.

3: 85 in the control and 86 in the Levucell® SC group.

In the first study, Charolais bull calves were stratified according to day of arrival and initial weight before being randomly allocated to pens.⁵¹ Treatments (control or Levucell® SC) were randomly allocated to pens (six per treatment). Animals were fed *ad libitum* a TMR. The additive was mixed with a protein concentrate and a mineral supplement and then top dressed on the TMR of the treated group. The concentration of yeast was confirmed in the protein concentrate, and then the concentration in the feed ingested was calculated to be 5×10^8 CFU/kg complete feed. The treatment effect on performance was tested using ANOVA. At the beginning of the trial, animals required intensive veterinary treatments due to adverse climatic conditions and no shelter.

In the second study, pens were then randomly allotted to one of the two groups (control or Levucell® SC), balanced for the initial BW of the heifers.⁵² A TMR with whole crop maize as the main forage ingredient was fed. Levucell® SC was added through a mineral–vitamin premixture, and yeast cells were determined both in the additive batch and in the premixture. The concentration of yeast in the feed was calculated to be 6×10^8 CFU/kg complete feed. Data were subjected to one-way ANOVA to test differences between treatments.

In the third study, individual feed intake was recorded using specific feeders and distinctive identification transponders.⁵³ The diet was a pelleted feed with concentrates mixed with straw and middlings as fibre sources. Levucell® SC was added through a middlings premixture. Yeast concentration was measured in the premixture (7×10^{11} CFU/kg) and in the feed (5×10^8 CFU/kg complete feed). Data were subjected to ANOVA, using initial BW as covariate and considering each animal as the experimental unit.

⁵⁰ Technical dossier FAD-2010-0120/Section IV/Annex IV_2_2.

⁵¹ Technical dossier FAD-2010-0120/Section IV/Annex IV_2_4.

⁵² Technical dossiers/Supplementary information October 2016/Annexes 10.

⁵³ Technical dossiers/Supplementary information October 2016/Annexes 11.

At the start of the fourth study, animals were weighed and randomly allocated in pens and these were randomly assigned to one of the two experimental groups (control or Levucell® SC) balanced for the initial BW.⁵⁴ Animals were fed a TMR with maize silage as the main forage ingredient, and Levucell® SC was added through a protein–mineral–vitamin premix, in which the yeast count was confirmed by analysis. From this value, it was calculated that the concentration of yeast in complete feed was 5×10^8 CFU/kg. Data were subjected to one-way ANOVA to test the differences between experimental treatments.

In three of the studies, a TMR with maize silage was the main forage ingredient and different concentrates were used to feed the animals (studies 1, 2 and 4). In these studies, feed intake was monitored by pen, and this was taken as the experimental unit. In study 3, a pelleted feed with concentrate, straw and middlings was used. In this latter study, animals were individually fed, and each animal was considered as the experimental unit. In all the studies growth performance was assessed by recording feed intake (per pen in studies 1, 2 and 4; or individual feed intake in study 3), initial and final body weight (BW) (with intermediate recordings in some studies). From these parameters, average daily gain (ADG) and feed to gain ratio were calculated. In studies 1, 2 and 4, carcass quality traits (weight, grading, dressing percentage) were monitored at the slaughter house.

The results of the four studies are summarised in Table 4.

Table 4: Effect of Levucell® SC on performance of cattle for fattening

Study	Levucell® SC (CFU/kg feed)	Initial weight (kg)	Final body weight (kg)	Average daily gain (kg)	Daily feed intake (kg DM/day)*	Feed:gain
1	0	334	653	1.52	8.6	5.75
	5×10^8	336	665	1.56	8.6	5.54
2	0	288	477	1.03 ^a	7.0	6.80
	6×10^8	317	525	1.13 ^b	7.3	6.56
3	0	177	479	1.57	7.8 ^b	5.01 ^b
	5×10^8	176	489	1.62	7.5 ^a	4.63 ^a
4	0	442	744**	1.60	10.4	6.48
	5×10^8	441	741**	1.68	11.0	6.60

CFU: colony forming unit.

*: In study 3, intake is for complete feed (as fed, 88% DM)

** : Animals were slaughtered at a pre-determined weight reached at 189 days for the control and 179 days for the Levucell® SC ($p < 0.10$)

a,b: Means within a column with different superscript letters are significantly different at $p < 0.1$

In study 2, the addition of Levucell® SC increased ADG significantly, with no effects on the other performance parameters. In study 3, there were no significant differences between groups in final BW and ADG, but feed to gain ratio was significantly improved in the Levucell® SC group compared with the control group. Effects of the additive on growth performance did not reach statistical significance in studies 1 and 4. But in study 4, animals were slaughtered at a similar final weight, and it was observed that steers of the Levucell® SC group reached the final weight with significant less days on fattening than the control animals (179 vs 189 days, $p = 0.08$, respectively).

Therefore, Levucell® SC at 8×10^9 CFU/head and day has the potential to improve the growth performance of cattle for fattening. This would approximate to a dose of 6×10^8 CFU/kg complete feedingstuffs.

3.3.4. Efficacy for minor ruminant species and camelids reared for fattening

Efficacy has been demonstrated for the major species (cattle for fattening). Given the physiological similarity (in gastrointestinal function and metabolism) between major and minor ruminant species, it can be reasonably assumed that the mechanism of action of the additive to be same. Therefore, data can be extrapolated between animals which are kept for the same purpose (in this case for fattening) at the dose of 6×10^8 CFU/kg complete feedingstuffs, without the need for species specific studies.

The applicant also proposes the use of the additive in growing camelids. There are anatomical differences in the gastrointestinal tract compared with ruminants, as camelids only have two pre-gastric

⁵⁴ Technical dossiers/Supplementary information October 2016/Annexes 12.

chambers (Reece et al., 2015). However, the strategy for digestion is essentially the same in camelids and ruminants, as in both there is a foregut pregastric microbial fermentation of feed (Fowler and Bravo, 2010; Reece et al., 2015). Therefore, the FEEDAP Panel accepts that data can be extrapolated to camelids kept for the same purpose (in this case for fattening) at the dose of 6×10^8 CFU/kg complete feedingstuffs, without the need for species specific studies.

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 Regulation⁵⁵ and Good Manufacturing Practice.

4. Conclusions

The FEEDAP Panel considers that the three available Levucell SC formulations are equivalent when used to deliver the same dose.

The active agent fulfils the requirements of the QPS approach to the assessment of safety and no concerns are expected from other components of the additive. Consequently, Levucell SC can be presumed safe for the target animals, consumers of products from treated animals and the environment.

Levucell SC is not a skin irritant or sensitiser but is an eye irritant. Inhalation exposure is unlikely. Encapsulation is not expected to introduce hazards for users.

The FEEDAP Panel cannot conclude on the efficacy of Levucell SC for dairy cows due to an insufficient number of positive studies. Consequently, no conclusion on minor dairy ruminant species or dairy camelids can be drawn.

Levucell SC has a potential to improve the performance of cattle raised for fattening when supplied at a minimum dose of 6×10^8 CFU/kg complete feed. This conclusion is extended to minor ruminant species and camelids reared for meat production at the same minimum concentration of 6×10^8 CFU/kg complete feed.

Documentation provided to EFSA

- 1) *Saccharomyces cerevisiae* CNCM I-1077 Levucell® SC. Application for the re-registration (under article 10 of Regulation (EC) No 1831/2003) of the use of Levucell SC 20, 10 ME and/or Titan in Beef Cattle and Dairy Cows. September 2010. Submitted by Lallemand S.A.S.
- 2) *Saccharomyces cerevisiae* CNCM I-1077. Application for the extension of use of Levucell SC 20/10 (ME) Titan to Calves, Buffaloes, and All (including Pseudo-) Ruminant species. November 2013. Submitted by Lallemand S.A.S.
- 3) Application for authorisation of Levucell SC20 and Levucell SC10 ME/Titan (*Saccharomyces cerevisiae* CNCM I-1077) for dairy cows and cattle for fattening and for minor ruminant species and camels submitted under Articles 10(2) and 4(1) of Regulation (EC) No 1831/2003. Supplementary Information. October 2016. Submitted by Lallemand S.A.S.
- 4) Application for authorisation of Levucell SC20 and Levucell SC10 ME/Titan (*Saccharomyces cerevisiae* CNCM I-1077) for dairy cows and cattle for fattening and for minor ruminant species and camels submitted under Articles 10(2) and 4(1) of Regulation (EC) No 1831/2003. Supplementary Information. March 2017. Submitted by Lallemand S.A.S.
- 5) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Levucell SC.
- 6) Comments from Member States.

References

EFSA (European Food Safety Authority), 2006a. Opinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the safety and efficacy of the product "Levucell SC20/Levucell SC10ME", a preparation of *Saccharomyces cerevisiae*, as a feed additive for dairy goats and dairy ewes in accordance with Regulation (EC) No 1831/2003. EFSA Journal 2006;4(6):370, 10 pp. <https://doi.org/10.2903/j.efsa.2006.370>

⁵⁵ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

- EFSA (European Food Safety Authority), 2006b. Opinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the safety and efficacy of the product "Levucell SC20/Levucell SC10ME", a preparation of *Saccharomyces cerevisiae*, as a feed additive for leisure horses. EFSA Journal 2006;4(9):385, 9 pp. <https://doi.org/10.2903/j.efsa.2006.385>
- EFSA (European Food Safety Authority), 2007. Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA - Opinion of the Scientific Committee. EFSA Journal 2007;5(11):587;16 pp. <https://doi.org/10.2903/j.efsa.2007.587>
- EFSA (European Food Safety Authority), 2008a. 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 Levucell SC20/Levucell SC10ME, a preparation of *Saccharomyces cerevisiae*, as feed additive for lambs for fattening. EFSA Journal 2008;6(7): 772, 9 pp. <https://doi.org/10.2903/j.efsa.2008.772>
- EFSA (European Food Safety Authority), 2008b. Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition. EFSA Journal 2008,6(9):803, 5 pp. <https://doi.org/10.2903/j.efsa.2008.803>
- EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on efficacy of the product Levucell SC20/Levucell SC10ME (*Saccharomyces cerevisiae*) as feed additive for leisure horses. EFSA Journal 2009;7(4):1040, 7 pp. <https://doi.org/10.2903/j.efsa.2009.1040>
- EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, Herman L, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Cocconcelli PS and Klein G (deceased), Prieto Maradona M, Querol A, Peixe L, Suarez JE, Sundh I, Vlak JM, Aguilera-Gomez M, Barizzone F, Brozzi R, Correia S, Heng L, Istace F, Lythgo C and Fernández Escámez PS, 2017. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. EFSA Journal 2017;15(3):4664, 177 pp. <https://doi.org/10.2903/j.efsa.2017.4664>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Technical guidance: Tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. <https://doi.org/10.2903/j.efsa.2011.2175>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. <https://doi.org/10.2903/j.efsa.2012.2536>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. <https://doi.org/10.2903/j.efsa.2012.2539>
- European Commission, 1997, updated 2003. Opinion on the use of certain micro-organisms as additives in feedingstuffs. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed_additives_rules_scan-old_report_out93.pdf
- Fowler ME and Bravo PW, 2010. Digestive System, in Medicine and Surgery of Camelids, 3rd Edition. Blackwell Publishing, Inc., Ames, Iowa, USA. <https://doi.org/10.1002/9781118785706.ch13>
- de Ondarza MB, Sniffen CJ, Dussert L, Chevaux E, Sullivan J and Walker N, 2010. CASE STUDY: Multiple-Study Analysis of the Effect of Live Yeast on Milk Yield, Milk Component Content and Yield, and Feed Efficiency. The Professional Animal Scientist, 26(2010), 661–666.
- Reece WO, Erickson HH, Goff JP and Uemura EE (eds), 2015. *Dukes' Physiology of Domestic Animals*, 13th Edition. Wiley-Blackwell, Oxford, UK.

Abbreviations

ANOVA	analysis of variance
ADG	average daily gain
BCS	body condition score
BW	body weight
CFU	colony-forming unit
CNCM	Collection Nationale de Cultures de Microorganismes
CV	coefficient of variation
DM	dry matter
EURL	European Union Reference Laboratory
LOQ	limit of quantification
PCR	polymerase chain reaction
QPS	Qualified Presumption of Safety
RH	relative humidity
SCAN	Scientific Committee on Animal Nutrition
TMR	Total Mixed Ration

Appendix A – Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Methods of Analysis for Levucell SC

In the current application authorisation is sought under article 10(2) for *Saccharomyces cerevisiae* CNCM I-1077 under the category / functional group 4(a) 'zootechnical additives'/ 'digestibility enhancers' and 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003.

The *feed additive* is intended to be marketed as a powder (Levucell SC 20) or in micro-encapsulated form (Levucell SC 10 ME and Levucell SC 10 ME Titan). The active substance of the *feed additive* are viable cells of *Saccharomyces cerevisiae* CNCM I-1077 at a minimum concentration of 1×10^9 CFU/g product. The strain is deposited at Collection Nationale de Cultures de Micro-organismes (C.N.C.M.) at Pasteur Institute.

The *feed additive* is intended to be used in *feedingstuffs* through *premixtures*. Specifically, the authorisation is sought for the use of the *feed additive* for dairy cows and beef cattle at a minimum dose of 4×10^8 and 5×10^8 CFU / kg *feedingstuffs*, respectively.

For the identification of *Saccharomyces cerevisiae* CNCM I-1077 the Applicant submitted the polymerase chain reaction (PCR) amplification method, a generally recognised methodology for microbial identification. This method was ring-trial validated to become the CEN technical standard (CEN/TS 15790:2008). The latter is recommended by EURL for official control to identify the *Saccharomyces cerevisiae* CNCM I-1077.

For the enumeration of *Saccharomyces cerevisiae* CNCM I-10077 in *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated CEN pour plate method for the enumeration of yeast probiotic strains (EN 15789), using yeast extract dextrose chloramphenicol agar (CGYE). The performance characteristics of the EN 15789 method 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 quantification (LOQ) of 1×10^5 CFU/kg, well below the minimum dose proposed by the Applicant.

Based on these performance characteristics the EURL recommends for official control, the CEN method EN 15789 for the enumeration of *Saccharomyces cerevisiae* CNCM I-1077 in *feed additive*, *premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.