Safety and efficacy of Levucell® SB (Saccharomyces cerevisiae CNCM I-1079) as a feed additive for chickens for fattening and minor poultry species

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Abstract

Levucell SB® is a feed additive consisting of viable cells of a strain of Saccharomyces cerevisiae currently authorised as a zootechnical additive for piglets and sows. The applicant is now seeking authorisation as a zootechnical additive (other zootechnical additive) for use with chickens for fattening and minor poultry species. The EFSA FEEDAP Panel in a previous opinion concluded that the additive fulfilled the requirements for the qualified presumption of safety (QPS) approach to safety assessment and thus could be presumed safe for the target species, consumers and the environment. Since the additive which is the subject of the present application has the same formulations as that considered previously, following the QPS approach, the same conclusions on target animal consumer and environmental safety apply when used with poultry. The use of the additive with diets for the new target species is considered unlikely to introduce hazards for users of the product not already considered in the previous assessment. When used with poultry feed, the additive is intended to aid the reduction of carcass contamination with enteropathogens and so improve the quality of poultry products. Five studies were submitted with chickens fed diets with or without the additive and then (in four studies) artificially challenged with Salmonella. The fifth study relied on the natural incidence of Salmonella. The prevalence of Salmonella contamination was estimated by examining the carcass directly and/or by detection in caecal, cloacal or faecal samples. Considering overall the results of the five studies, there is evidence that the addition of Levucell SB® to diets at a dose of $2 \times 10^{10}$ CFU/kg feed can aid the reduction of carcass contamination with Salmonella spp.. This conclusion can be extrapolated to minor avian species for fattening when used at the same dose, but not to minor poultry species for laying.

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Keywords: Levucell SB, Saccharomyces cerevisiae CNCM I-1079, chickens, minor poultry species, efficacy, Salmonella, Campylobacter

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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.

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1. **Introduction**

1.1. **Background and Terms of Reference**

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

The European Commission received a request from Lallemand SAS\(^2\) for authorisation of the product Levucell SB\(^5\) (Saccharomyces cerevisiae CNCM I-1079), when used as a feed additive for chickens for fattening and minor poultry species (category: zootechnical additive; functional group: other zootechnical additive).

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 dossiers in support of these applications. The particulars and documents in support of the application were considered valid by EFSA as of 24 August 2015.

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 animals, consumer, user and the environment and on the efficacy of the product Levucell SB\(^5\) (Saccharomyces cerevisiae CNCM I-1079), when used under the proposed conditions of use (see Section 3.2).

1.2. **Additional information**

The additive Levucell SB\(^5\) is a preparation of *S. cerevisiae* (CNCM I-1079). The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the safety and efficacy of this product when used with piglets and sows (EC 1997, updated 2003). Subsequently, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) produced an opinion on the re-evaluation of Levucell SB\(^5\) when used with the same target species (EFSA FEEDAP Panel, 2016). The additive is currently authorised for use with piglets\(^3\) and sows.\(^4\)

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, 2013). This approach requires the identity of the strain to be established.

2. **Data and methodologies**

2.1. **Data**

The present assessment is based on data submitted by the applicant in the form of a technical dossier\(^5\) in support of the authorisation request for the use of Levucell SB\(^5\) (Saccharomyces cerevisiae CNCM I-1079) as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008\(^6\) and the applicable EFSA guidance documents.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.\(^7\)

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\(^1\) 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.

\(^3\) Commission Regulation (EC) No 1847/2003 of 20 October 2003 concerning the provisional authorisation of a new use of an additive and the permanent authorisation of an additive already authorised in feedingstuffs. OJ L 269, 21.10.2003, p. 3.

\(^4\) Commission Regulation (EC) No 2036/2005 of 14 December 2005 concerning the permanent authorisations of certain additives in feedingstuffs and the provisional authorisation of a new use of certain additives already authorised in feedingstuffs. OJ L 328, 15.12.2005, p. 13.

\(^5\) FEED dossier reference: FAD-2015-0017.

\(^6\) 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.

\(^7\) The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/fnrep-fad-2010-0121-levucell.pdf
2.2. Methodologies

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

3. Assessment

The additive is a preparation consisting of dried cells of *S. cerevisiae* (CNCM I-1079) intended for use as a zootechnical additive (other zootechnical additive) in feed for chickens for fattening and minor poultry species to aid the reduction of carcass contamination with enteropathogens and so to improve the quality of poultry products.

3.1. Characterisation of the additive

The additive which is the subject of the present application has the same formulations and method of manufacture as that considered in a previous application (EFSA FEEDAP Panel, 2016). It is marketed in three formulations: Levucell® SB20 with a minimum concentration of $2 \times 10^{10}$ colony-forming units (CFU)/g viable yeast cells, and Levucell® SB10 ME and Levucell® SB10 ME TITAN, both microencapsulated forms (which differ minimally owing to a slightly different drying process) with a minimum concentration of $1 \times 10^{10}$ CFU/g viable yeast cells. For practical purposes, the two coated forms are considered equivalent. The data pertaining to impurities, physical properties and stability, submitted in the previous application dossier, still apply. However, the applicant has submitted additional data on the stability of the additive and its capacity to mix with poultry feed.

The stability of Levucell® SB20 and SB10 ME TITAN was monitored for a period of 6 months when incorporated into a vitamin-mineral premix for chickens for fattening and stored at 20°C. Loss of viability was < 0.5 log for both formulations.

Three batches of SB10 ME TITAN were used to prepare mash feed for chickens for fattening at an intended inclusion rate of $1 \times 10^9$ CFU/kg feed. Subsamples of the same feed were subsequently pelleted (74–78°C). Both mash and pelleted feeds were then stored for 6 months at 20°C with counts of viable yeasts made at monthly intervals. Essentially, no loss in viability was seen in either the mash or pelleted feed over the period monitored. The same results were seen with Levucell® SB20 when three batches were tested under the same conditions. The process of pelleting feed containing the TITAN formulation under the conditions described resulted in a small loss in viability (average of a 0.46 reduction in log count).

The ability of Levucell® SB20 and SB10 ME TITAN to be uniformly mixed into feed was examined using a single batch of the additive mixed into pelleted and/or mash feed for chickens at an inclusion rate of $1 \times 10^9$ CFU/kg. Analyses of counts made with 10 subsamples of each feed showed a coefficient of variation of 1–2% for both forms of the additive.

3.2. Conditions of use

The product is intended for use in feed for chickens for fattening and minor poultry species at a minimum dose of $1 \times 10^9$ CFU/kg complete feedingstuffs irrespective of the formulation used.

3.3. Safety

In an opinion on the use of Levucell® SB in feed for piglets and sows, the FEEDAP Panel concluded that Levucell® SB meets the requirements for the QPS safety assessment, and therefore *Saccharomyces cerevisiae* CNCM I-1079 can be presumed safe for target species, consumers of product derived from animals fed the additive and the environment (EFSA FEEDAP Panel, 2016).

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8 Technical dossier/Section II/Annex II_1_5.
9 Technical dossier/Section II/Annexes II_4_9.
10 Technical dossier/Section II and Supplementary information April 2016/Annexes II_4_9-12 and 3.
11 Technical dossier/Section II/Annex II_4_13.
12 Technical dossier/Supplementary information April 2016/Annexes 1 and 2.
In the context of the same opinion, it was concluded that Levucell® SB20 (the uncoated form) is not a skin or eye irritant or a skin sensitisser and that inhalation exposure is unlikely. Furthermore, the encapsulation used in the two other forms (Levucell® ME10 and Levucell® ME TITAN) is not expected to introduce hazards for users. The use of the additive with diets for chickens for fattening and minor poultry species is considered unlikely to introduce hazards for users of the product not already considered.

3.4. Efficacy

The proposed purpose of the additive differs from previous applications. When used in feed for poultry, it is intended to aid the reduction of carcass contamination with enteropathogens and so improve the quality of poultry products. The various studies submitted were conducted with Levucell® SB20 except study 4 which was made with Levucell® SB10 ME TITAN. 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.

3.4.1. Efficacy for chickens for fattening

Five studies made with chickens for fattening, four performed in different Member States and one outside the European Union (EU), are described in the dossier. Two of them are published studies (Al-Zenki et al., 2009; Fanelli et al., 2015). One of these is presented below. In all studies, a control group receiving a basal diet was compared with at least one group receiving the same basal diet supplemented with Levucell® SB at the minimum recommended dose (1 × 10⁹ CFU/kg feed), except one study that included a higher dose. All doses were confirmed by analysis. All of the birds on trial were then challenged with strains of the Salmonella and/or Campylobacter. When designing four of these trials, the applicant took account of the published EFSA Opinions which concluded that the incidence of Campylobacter and Salmonella contamination of poultry meat is indicated by the amounts carried on the neck and or breast skin (EFSA BIOHAZ Panel, 2011a,b). The methods used for sampling and subsequent microbiological examinations are provided for each trial.

One of the publications to which the applicant drew the attention to regarded a study examining the addition of Levucell® SB20 to the diets of chickens at different seasons with the intention of reducing the natural incidence of contamination by Salmonella (Al-Zenki et al., 2009). In this study, Levucell® SB was included as one of a number of agents tested for their ability to reduce naturally occurring carcass contamination by Salmonella. Each arm of the study (summer with an average temperature of 28°C and winter with an average temperature of 23°C) consisted of nine replicate pens of 120 1-day-old chicks (breed not specified) including an untreated control group. Levucell® SB20 was supplied via diet at 1 g/kg feed which would equate to a minimum of 2 × 10¹⁰ CFU/kg feed. Although the overall study was analysed by an analysis of variance, the Tukey test was used to separate treatment means allowing the Levucell data to be directly compared to the control data. Birds received a maize/soybean diet for 35 days, the duration of the experiment. The entire experiment was repeated twice, once during the summer season and once in the winter. Litter samples (25/pen pooled) were taken on days 7, 21 and 35 at the farm. The presence of Salmonella was determined using five birds/pen by washing the full carcass with buffered peptone water on farm on days 7, 21 and 35 and on carcass samples after slaughter (post-chilling, five/pen). Caecal samples were also obtained from the same birds used to estimate the prevalence of Salmonella.

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13 This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
14 Technical dossier/Section IV/Annexes IV_1_1, IV_1_2, IV_1_3 and Supplementary information April 2016/Annexes 6 and 7.
The presence of Levucell® SB20 in the diet significantly reduced the incidence of *Salmonella* on carcasses and in the caecum (Table 1) at each of the three summer sampling periods and at the slaughterhouse. Differences were less evident during the winter period and reached significance only for the final sampling on farm. Treatment with Levucell® SB20 had no adverse effect on any of the production parameters that were measured.

The applicant submitted two further published studies involving the addition of Levucell® SB20 to the diets of chickens with the intention of reducing contamination by *Salmonella* or *Campylobacter*. The first study evaluated the additive’s ability to reduce these bacterial populations in the caecum of chickens subjected to transport stress (Line et al., 1997). Birds, inoculated with individual strains of *Salmonella* and *Campylobacter* were held for 6 weeks and then divided into two groups (of two pens each) with half of the chickens receiving 10% dried yeast in feed for 60 h (equivalent to $2 \times 10^{10}$ CFU/kg feed). The birds were then caged and transported to simulate commercial conditions. With no yeast treatment, transport stress caused the *Salmonella* colonisation frequency to increase from 3.3% to 16.7%. No *Salmonella* were recovered from the caecum of those birds given Levucell® SB20. Similar results for *Salmonella* were obtained from birds challenged with a mixture of *Salmonella* and *Campylobacter* strains. The frequency of *Campylobacter* isolation was not affected by treatment, but *Campylobacter* populations present in the caecum were significantly reduced in the mixed strain trial.

The second study was conducted by the same group (Line et al., 1998) and investigated the effect of Levucell® SB20 on the caecal colonisation of chickens for fattening with *Salmonella Typhimurium* and *C. jejuni* when housed in cages. Duplicate cages of broiler chicks (10 birds/replicate) were given *ad libitum* access to a standard feed supplemented with no yeast (control), or 1 or 100 g dried yeast/kg feed (equivalent to $2 \times 10^{10}$ CFU/kg feed and $2 \times 10^{12}$ CFU/kg feed, respectively). All chicks except negative controls were challenged on day 4 with $3.2 \times 10^8$ CFU *S. Typhimurium* and $6.5 \times 10^8$ CFU *C. jejuni* by oral gavage. After 23 days, the caecum was aseptically removed and analysed for *Salmonella* and *Campylobacter*. Frequency of *Salmonella* colonisation and counts in positive samples were reduced by both yeast treatments. *Campylobacter* colonisation was not significantly affected by yeast treatment. Similar results were obtained from a second trial conducted in larger floor pens. However, the limited replication in both studies did not allow a statistical assessment to be made on a cage/pen basis.

### Table 1: Effects of Levucell® SB20 on *Salmonella* prevalence (%) in caecal contents and on chickens’ carcass at 7, 21 and 35 days after challenge and post-chilling

| Treatment | Summer | | | Winter | | | |
|-----------|--------|--------|--------|--------|--------|--------|--------|
|           | Day 7  | Day 21 | Day 35 | Post-chill | Day 7 | Day 21 | Day 35 | Post-chill |
| Carcass contamination | | | | | | | | |
| Control   | 64.4 a | 71.1 a | 51.1 a | 26.7 a | 35.6 | 46.7 | 26.7 a | 2.2 |
| Levucell  | 22.2 b | 33.3 b | 20.0 b | 4.4 b | 13.0 | 35.6 | 4.4 b | 0 |
| Caecal contents | | | | | | | | |
| Control   | 73.3 a | 64.4 a | 55.6 a | 28.9 a | 35.6 | 46.7 | 35.6 a | 15.6 |
| Levucell  | 44.4 b | 33.3 b | 11.1 b | 6.7 b | 22.2 | 33.3 | 8.9 b | 8.9 |

a,b: Means within a column with different superscript letters are significantly different ($p < 0.05$).

The presence of Levucell® SB20 in the diet significantly reduced the incidence of *Salmonella* on carcasses and in the caecum (Table 1) at each of the three summer sampling periods and at the slaughterhouse. Differences were less evident during the winter period and reached significance only for the final sampling on farm. Treatment with Levucell® SB20 had no adverse effect on any of the production parameters that were measured.

The applicant submitted two further published studies involving the addition of Levucell® SB20 to the diets of chickens with the intention of reducing contamination by *Salmonella* or *Campylobacter*. The first study evaluated the additive’s ability to reduce these bacterial populations in the caecum of chickens subjected to transport stress (Line et al., 1997). Birds, inoculated with individual strains of *Salmonella* and *Campylobacter* were held for 6 weeks and then divided into two groups (of two pens each) with half of the chickens receiving 10% dried yeast in feed for 60 h (equivalent to $2 \times 10^{10}$ CFU/kg feed). The birds were then caged and transported to simulate commercial conditions. With no yeast treatment, transport stress caused the *Salmonella* colonisation frequency to increase from 3.3% to 16.7%. No *Salmonella* were recovered from the caecum of those birds given Levucell® SB20. Similar results for *Salmonella* were obtained from birds challenged with a mixture of *Salmonella* and *Campylobacter* strains. The frequency of *Campylobacter* isolation was not affected by treatment, but *Campylobacter* populations present in the caecum were significantly reduced in the mixed strain trial.

The second study was conducted by the same group (Line et al., 1998) and investigated the effect of Levucell® SB20 on the caecal colonisation of chickens for fattening with *Salmonella Typhimurium* and *C. jejuni* when housed in cages. Duplicate cages of broiler chicks (10 birds/replicate) were given *ad libitum* access to a standard feed supplemented with no yeast (control), or 1 or 100 g dried yeast/kg feed (equivalent to $2 \times 10^{10}$ CFU/kg feed and $2 \times 10^{12}$ CFU/kg feed, respectively). All chicks except negative controls were challenged on day 4 with $3.2 \times 10^8$ CFU *S. Typhimurium* and $6.5 \times 10^8$ CFU *C. jejuni* by oral gavage. After 23 days, the caecum was aseptically removed and analysed for *Salmonella* and *Campylobacter*. Frequency of *Salmonella* colonisation and counts in positive samples were reduced by both yeast treatments. *Campylobacter* colonisation was not significantly affected by yeast treatment. Similar results were obtained from a second trial conducted in larger floor pens. However, the limited replication in both studies did not allow a statistical assessment to be made on a cage/pen basis.

### 3.4.1.1. Conclusions on efficacy for chickens for fattening

The Panel recognises that challenge experiments present technical difficulties. Considering the limitations (e.g., poor description of the challenge strains, use of limited number of serotypes, housing of birds not reflecting commercial conditions and potentially impacting carcass contamination) and overall the results of the five studies, there is some evidence that the addition of Levucell® SB to diets has a potential to aid the reduction of carcass contamination with *Salmonella* spp. and so improve the quality of poultry products. The effective dose appears to be $2 \times 10^{10}$ CFU/kg feed. Insufficient data were provided to conclude on the possible effects of Levucell® SB on carcass contamination by *Campylobacter* spp..

### 3.4.2. Efficacy for minor poultry species

Since the applicant proposes the use of the same dose in minor avian species and as the mechanism of action of the additive can be reasonably assumed to be same, Levucell® SB can be presumed efficacious for the reduction of carcass contamination by *Salmonella* in minor avian species.
for fattening at a dose of $2 \times 10^{10}$ CFU/kg feed without the need for additional studies. However, in the absence of data, no conclusions can be drawn on the efficacy for minor poultry species for laying.

4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation\textsuperscript{15} and Good Manufacturing Practice.

5. Conclusions

The active agent fulfils the requirements of the QPS approach to the assessment of safety and can be presumed safe for target animals, consumers of products from treated animals and the environment when used in feed for chickens for fattening and for minor poultry species.

The use of the additive with feed for chickens for fattening and for minor poultry species is considered unlikely to introduce hazards for users of the product not already considered in previous assessments on Levucell SB\textsuperscript{®}.

The FEEDAP Panel considers that the three available formulations are equivalent when used to deliver the same dose. The addition of Levucell\textsuperscript{®} SB to diets for chickens for fattening at a dose of $2 \times 10^{10}$ CFU/kg feed can aid the reduction of carcass contamination with \textit{Salmonella} spp. and so improve the quality of poultry products. This conclusion can be extrapolated to minor poultry species for fattening when used at the same dose, but not to minor poultry species for laying.

Documentation provided to EFSA

1) Levucell\textsuperscript{®} SB for chickens for fattening and minor poultry species. April 2015. Submitted by LALLEMAND SAS.
2) Levucell\textsuperscript{®} SB for chickens for fattening and minor poultry species. Supplementary information. April 2016. Submitted by LALLEMAND SAS.
3) Comments from the Member States.

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\textsuperscript{15} Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.
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**Abbreviations**

ANOVA  analysis of variance
ATCC  American Type Culture Collection
CFU  colony-forming unit
EUURL  European Union Reference Laboratory
FEEDAP  EFSA Panel on Additives and Products or Substances used in Animal Feed
GLM  generalized linear model
QPS  Qualified Presumption of Safety
SCAN  Scientific Committee on Animal Nutrition