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Assessment of the application for renewal of authorisation of GalliPro[®] (*Bacillus subtilis* DSM 17299) for chickens for fattening

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Abstract

GalliPro[®] is the trade name for a feed additive based on viable cells of a strain of *Bacillus subtilis* intended for use as a zootechnical additive (gut flora stabiliser) in feed for chickens for fattening. The product is currently authorised for use in chickens for fattening. This opinion concerns the renewal of this authorisation. *Bacillus subtilis* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to establish the safety for the target species, consumers and the environment. The identity of the strain present in the additive was established and evidence was provided on the lack of toxigenic potential as well as acquired antibiotic resistance determinants to antibiotics of human and veterinary importance. Accordingly, this strain is presumed safe for the target species, consumers of products derived from animals fed the additive and the environment. Since no concerns are expected from other components of the additive, GalliPro[®] is considered safe for the target species, consumers and the environment. The applicant has provided evidence that the additive currently in the market complies with the existing conditions of authorisation. The FEEDAP Panel confirms its previous conclusions that GalliPro[®] is safe for the target species; consumers of products from animals fed the additive and the environment. GalliPro[®] should be considered a potential respiratory sensitiser. In the absence of data, the FEEDAP Panel cannot conclude on the potential of GalliPro[®] for skin and eyes irritancy and dermal sensitisation.

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Keywords: zootechnical additive, gut flora stabiliser, GalliPro[®], *Bacillus subtilis* DSM 17299, safety, QPS, chickens for fattening

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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 14 of that Regulation specifies that for products authorised according to Article 9, an application for renewal shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation.

The European Commission received a request from Chr. Hansen A/S² for renewal of the authorisation of the product GalliPro^{®3} (*Bacillus subtilis* DSM 17299), when used as a feed additive for chickens for fattening (category: zootechnical additive; functional group: gut flora stabiliser).

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 14(1) (renewal of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 7 December 2016.

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 the product GalliPro[®] (*Bacillus subtilis* DSM 17299), when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

EFSA has issued several opinions on the safety and efficacy of GalliPro[®] for chickens for fattening and on its compatibility with coccidiostats (EFSA, 2006, 2007a, 2008, 2009, 2010). In 2011, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) produced an opinion on the modification to the formulation of GalliPro[®] and on its compatibility with formic acid (EFSA FEEDAP Panel, 2011).

The product is currently authorised for use in chickens for fattening.⁵ It was originally authorised under the tradename O35 as a product containing a minimum guaranteed concentration of *Bacillus subtilis* DSM 17299 of 1.6×10^9 colony forming units (CFU) per gram of additive, intended for use in feed for chickens for fattening at a minimum concentration of 8×10^8 CFU/kg feed and a maximum concentration of 1.6×10^9 CFU/kg feed. In 2011, the authorisation was amended to increase the minimum guaranteed concentration of *Bacillus subtilis* DSM 17299 to 1.6×10^{10} CFU/g of additive and to allow the simultaneous use with formic acid. In an amendment in 2012, the maximum concentration in feed was removed. Other amendments in 2010 and 2011 regarded the authorisation of use in the presence of the coccidiostats diclazuril, halofuginone, robenidine, decoquinat, narasin/nicarbazin, lasalocid sodium, maduramycin ammonium, monensin sodium, narasin, salinomycin sodium, semduramycin sodium.

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 Chr. Hansen A/S, 10-12 Boege Allé, DK 2970, Hoersholm, Denmark.

³ The Applicant also markets the product under other trade names: O35, GalliPro[®] Max, GalliPro[®] 10, [REDACTED]

⁵ Commission Regulation (EC) No 1137/2007 of 1 October 2007 concerning the authorisation of *Bacillus subtilis* (O35) as a feed additive. OJ L 256, 2.10.2007, p. 5; Commission Regulation (EC) No 203/2009 of 16 March 2009 amending Regulation (EC) No 1137/2007 as regards the use of the feed additive *Bacillus subtilis* (O35) in feed containing decoquinat and narasin/nicarbazin, OJ L 71, 17.3.2009, p. 11; Commission Regulation (EU) No 515/2010 of 15 June 2010 amending Regulation (EC) No 1137/2007 as regards the use of the feed additive *Bacillus subtilis* (O35) in feed containing lasalocid sodium, maduramycin ammonium, monensin sodium, narasin, salinomycin sodium and semduramycin sodium, OJ L 150, 16.6.2010, p. 44; Commission Implementing Regulation (EU) No 881/2011 of 2 September 2011 amending Regulation (EC) No 1137/2007 as regards the additive composition of the preparation of *Bacillus subtilis* DSM 17299 (holder of authorisation Chr. Hansen A/S) and its use in feed containing formic acid, OJ L 228, 3.9.2011, p. 9; COMMISSION IMPLEMENTING REGULATION (EU) No 1018/2012 of 5 November 2012 amending Regulations (EC) No 232/2009, (EC) No 188/2007, (EC) No 186/2007, (EC) No 209/2008, (EC) No 1447/2006, (EC) No 316/2003, (EC) No 1811/2005, (EC) No 1288/2004, (EC) No 2148/2004, (EC) No 1137/2007, (EC) No 1293/2008, (EC) No 226/2007, (EC) No 1444/2006, (EC) No 1876/2006, (EC) No 1847/2003, (EC) No 2036/2005, (EC) No 492/2006, (EC) No 1200/2005, and (EC) No 1520/2007 as regards the maximum content of certain micro-organisms in complete feedingstuffs; OJ L 307, 7.11.2012, p. 56.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁶ in support of the request for the renewal of the authorisation for the use of GalliPro® (*Bacillus subtilis* DSM 17299) as a feed additive.

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

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of GalliPro® (*Bacillus subtilis* DSM 17299) is in line with the principles laid down in Regulation (EC) No 429/2008⁸ and the relevant guidance documents: Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012), Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2013) and Guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition (EFSA FEEDAP Panel, 2014).

3. Assessment

The additive GalliPro® consists of viable spores of *Bacillus subtilis* DSM 17299 and is currently authorised as a zootechnical additive (gut flora stabiliser) in feed for chickens for fattening. The applicant is asking for the renewal of the authorisation.

3.1. Characterisation

3.1.1. Characterisation of the additive

GalliPro® is currently authorised as a solid preparation with a minimum guaranteed concentration of *B. subtilis* DSM 17299 of 1.6×10^{10} CFU/gram of additive.⁹

The additive is composed of 3% spores concentrate, 96% carrier (limestone/calcium carbonate) and 1% of anticaking agent (Kieselgur). The applicant stated that no changes in the manufacturing process or composition of the additive have been introduced since the last authorisation. Compliance with specifications was confirmed by the analysis of three recent batches produced (range: $2.2\text{--}2.3 \times 10^{10}$ CFU/g, average: 2.3×10^{10} CFU/g).¹⁰

Data from the same batches confirmed compliance with specifications for total coliforms ($< 10^3$ CFU/g), *Escherichia coli* (< 10 CFU/g), yeasts and filamentous fungi ($< 10^3$ CFU/g), and *Salmonella* spp. (absent in 25 g).¹⁰ Data from other three recent batches confirmed compliance with specifications for undesirable substances. Values were: aflatoxin B1 (< 0.64 µg/kg),¹¹ mercury (0.0106–0.0146 mg/kg, lead (0.458–0.850 mg/kg), cadmium (0.033–0.092 mg/kg), arsenic (0.535–0.892 mg/kg).¹²

The dusting potential of three batches of the additive was tested with a Heubach dustmeter and showed values in the range $2.3\text{--}2.5$ g/m³.¹³

⁶ FEED dossier reference: FAD-2016-0059.

⁷ The full report is available on the EURL website: <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2005-0021?search&form-return>

⁸ 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.

⁹ Technical dossier/Section II.

¹⁰ Technical dossier/Section II/Annex II.1.3b.

¹¹ Limit of quantification.

¹² Technical dossier/Section II/Annex II.1.4.1.

¹³ Technical dossier/Supplementary information July 2018/Annex II.1.5b.v2.

3.1.2. Characterisation of the active agent

The active agent of GalliPro® is deposited in the German Collection of Microorganisms and cell Cultures with the accession number DSM 17299.¹⁴

Strain identification was achieved by pulsed-field gel electrophoresis (PFGE) after cleavage with restriction enzymes. The lack of resistance to relevant antibiotics and of toxigenic potential have been demonstrated following the guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012) and the guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition (EFSA FEEDAP Panel, 2014).

3.1.3. Conditions of use

GalliPro® is currently authorised in feed for chickens for fattening at the minimum inclusion level of 8×10^8 CFU/kg complete feedingstuff.

It is allowed to be used simultaneously with the coccidiostats: diclazuril, halofuginone, robenidine, decoquinate, narasin/nicarbazin, lasalocid sodium, maduramycin ammonium, monensin sodium, narasin, salinomycin sodium and semduramycin sodium, and with formic acid.

The applicant proposes to maintain the same conditions of use.

3.2. Safety

3.2.1. Safety for the target species, consumers and the environment

The bacterial species *B. subtilis* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007a,b; EFSA BIOHAZ Panel, 2017). This approach requires the identity of the active agent to be established and evidence that the strain lacks of toxigenic potential and does not show resistance to antibiotics of human and veterinary importance. The identity of the GalliPro® strain was established as *B. subtilis* and the lack of toxigenic potential and of acquired antibiotic resistance determinants to relevant antibiotics was established. Accordingly, this strain is considered by EFSA to be suitable for the QPS approach to safety and is presumed safe for the target species, consumers of products derived from animals fed the additive and the environment. Since no concerns are expected from other components of the additive, GalliPro® is also considered safe for the target species, consumers of products from animals fed the additive and the environment.

3.2.2. Safety for the user

In its original opinion (EFSA, 2006), the FEEDAP Panel concluded that the additive due to its proteinaceous nature may induce respiratory sensitisation.

The dustiness of the preparation tested indicated a potential for users to be exposed via inhalation. No additional information has been provided in this application. No data are available on skin/eye irritation or skin sensitisation. In the absence of data, the FEEDAP Panel cannot conclude on the potential of GalliPro® for skin and eyes irritancy and dermal sensitisation.

3.2.3. Further evidence

The applicant declares to have a post-market monitoring system in place and that no adverse effects or complaints have been reported by users of the product or workers handling it during production process.¹⁹

The applicant conducted a literature search on the safety of GalliPro® using several databases: Academic Onefile, Food Science Source, Agris and PubMed.²⁰ The search included the terms:

¹⁴ Technical dossier/Supplementary information July 2018/Annex II.2.1.2a.v2.

¹⁹ Technical dossier/Section III.

²⁰ Technical dossier/Supplementary information July 2018/Annexes III.3 and Supplementary information October 2018.

Bacillus subtilis, DSM 17299, ██████████, GalliPro, ██████████, safety, toxicity and adverse effect. The search covered the period 2007–2018 and identified 67 potential relevant publications (Appendix A). The majority of the experiments were not designed to assess the safety *per se* of the additive, but to investigate the effect(s) of the additive on zootechnical performance. Some included also health parameters (i.e. blood biochemistry, mortality), and one in particular investigated the immune response of *Salmonella* challenged chickens fed diets containing GalliPro®. The results did not evidence any adverse effect of the additive on the immune response of birds, either in challenged or non-contaminated birds (Sadeghi et al., 2015). A second study tested the effects of the supplementation of an additive containing the strain under assessment ██████████ on tibial bone characteristics in chickens (e.g. thickness of the medial and lateral wall of the tibia, phosphorous content) ██████████. Results did not reveal any adverse effect on the parameters measured.

Therefore, the FEEDAP Panel concludes that there is no new evidence that would lead the Panel to reconsider its previous conclusions on the safety of the product for target species, consumers, users and the environment under the authorised conditions of use.

3.2.4. Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the conditions of the original authorisation that would have an impact on the efficacy of the additive. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

3.3. 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 Regulation²¹ and Good Manufacturing Practice.

4. Conclusions

The applicant has provided evidence that the additive currently in the market complies with the existing conditions of authorisation.

The FEEDAP Panel confirms its previous conclusions that GalliPro® is safe for the target species, consumers of products from animals fed the additive and the environment. GalliPro® should be considered a potential respiratory sensitiser. In the absence of data, the FEEDAP Panel cannot conclude on the potential of GalliPro® for skin and eyes irritancy and dermal sensitisation.

Documentation provided to EFSA/Chronology

Date	Event
29/09/2016	Dossier received by EFSA. GalliPro. Submitted by Chr. Hansen A/S
24/10/2016	Reception mandate from the European Commission
07/12/2016	Application validated by EFSA – Start of the scientific assessment
07/03/2017	Comments received from Member States
10/10/2017	Spontaneous submission of information by the applicant. <i>Issues: safety for the consumer</i>
05/02/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation, safety for target species, consumer, user and environment</i>
19/07/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
12/10/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended <i>Issues: Safety</i>
29/10/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
01/04/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

²¹ 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.

Appendix A – List of references retrieved from the literature search provided by the applicant to support safety of the additive

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