REGULATED PRODUCTS SAFETY ASSESSMENT

Assessment on Safety and Modification of the Terms of a Feed Additive Consisting of *Bacillus Subtilis* DSM 32324, *Bacillus Subtilis* DSM 32325 and *Bacillus Amyloliquefaciens* DSM 25840 (GalliPro® Fit) for All Poultry Species for Fattening and Reared for Laying/breeding (Chr. Hansen A/S) (RP2019)

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FSA Research and Evidence

An application was submitted to the Food Standards Agency in April 2023 from Chr. Hansen A/S ("the applicant") for the modification to the authorisation of an additive consisting of *Bacillus subtilis* DSM 32324, *Bacillus subtilis* DSM 32325 and *Bacillus amyloliquefaciens* DSM 25840 (GalliPro® Fit), under the category of 'zootechnical additive' and functional group 'gut flora stabilisers' for its use in feed and water in all poultry species for fattening and reared for laying or for breeding. Modification to the current conditions of use of the additive is sought to allow simultaneous use with the approved coccidiostats monensin, salinomycin, narasin, nicarbazin+narasin and lasalocid.

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) concluded that *Bacillus subtilis* DSM 32324, *Bacillus subtilis* DSM 32325 and *Bacillus amyloliquefaciens* DSM 25840 (GalliPro® Fit) remains safe for the target species, consumers and the environment. The Panel concluded that the additive is not an irritant to the skin and eyes but should be considered a respiratory sensitiser. Skin sensitisation could not be concluded upon. The Panel concluded that the additive is compatible with the coccidiostats monensin, salinomycin, narasin, nicarbazin+narasin and lasalocid.

The FSA/FSS has reviewed the applicant's modification to the authorisation application, supporting documentation, and other regulators risk assessments, most notably the EFSA risk assessment opinion, and considers that sufficient evidence has been demonstrated to conclude without the need for further questions or risk assessment.

This is a joint FSA and FSS publication.

1. Introduction

The FSA and FSS have undertaken an assessment of a feed additive (Chr. Hansen A/S, 10-12 Boege Allé, DK-2970, Hoersholm, Denmark) consisting of *Bacillus subtilis* DSM 32324, *Bacillus subtilis* DSM 32325 and *Bacillus amyloliquefaciens* DSM 25840 (GalliPro® Fit) under Assimilated Regulation (EC) No 1831/2003 (EC, 2003) in each nation of Great Britain (GB) for a modification to current authorisation under category of 'zootechnical additive' and functional group 'gut flora stabilisers' for use in feed and water in all poultry species for fattening and reared for laying or for breeding. Under Assimilated Commission Implementing Regulation (EU) No 2020/1762 (EC, 2020), the additive is currently authorised for use in feed and water for all poultry species for fattening or reared for laying or reared for breeding.

In line with Article 8 of Assimilated Regulation (EC) No 1831/2003, the assessment has considered and concluded the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by the European Food Safety Authority (EFSA) for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

To ensure regulatory systems of FSA/FSS are risk proportionate and resources are used effectively, the FSA and FSS have used the evidence submitted by the applicant and other information in the public domain, including the EFSA risk assessment opinion, to provide a summary assessment of the evidence of safety presented in this report.

In 2023, EFSA published a risk assessment opinion (EFSA, 2023) on the modification of the terms of authorisation of *B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840 as a feed additive for all poultry species for fattening and reared for laying/breeding (Chr. Hansen A/S). This opinion has been reviewed by FSA/FSS risk assessors. It has been verified that the standard approach taken, when compared to the relevant guidance applied in GB, has been followed and the conclusions made are consistent with the data summarised in the opinion.

The result of the assessment is that there is sufficient evidence of safety for the UK to conclude this assessment at this time. This assessment represents the opinion of the FSA and FSS.

Table 1. Table showing products included in this assessment

Title	Product type	Intended use/s	Intended dose/intake
Bacillus subtilis DSM 32324, *Bacillus subtilis* DSM 32325 and *Bacillus amyloliquefaciens* DSM 25840	Feed additive	Zootechnical	• 1.6×10^9 CFU/kg complete feed or; • 5.4×10^8 CFU/L of water for drinking

2. Assessment

2.1. Detail of other regulators opinions

2.1.1. Previous authorisations and opinions

In 2020, the FEEDAP Panel concluded that *B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840 (GalliPro® Fit) is safe for the target species, the consumer and the environment. All three of the strains were considered suitable for the qualified presumption of safety (QPS) approach to assessment, with the identity of the active substances confirmed and the lack of toxigenic potential demonstrated (EFSA, 2020). Owing to the absence of data, the Panel were unable to conclude on the skin and eye irritation and skin sensitisation potential of the additive. Due to its proteinaceous nature the additive was considered to be a respiratory sensitiser.

The Panel concluded that the additive is compatible with diclazuril, decoquinate and halofuginone, however, owing to lack of data, the additive's compatibility with other coccidiostats could not be determined. The additive has the potential to be efficacious in chickens for fattening at 1.6 x 10^9 CFU/kg feed and at 5.4 x 10^8 CFU/L in drinking water. This was extrapolated to all other poultry species for fattening or reared for laying/ breeding.

2.1.2. Methodology applied in the EFSA opinion

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) assessed the safety and the efficacy of *B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840 (GalliPro® Fit), in accordance with guidance documents:

- Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017);
- Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a);
- Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b);
- EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (EFSA, 2021);

and principles in Assimilated Regulation (EC) No 429/2008 (EC, 2008).

These guidance documents were developed and implemented prior to the UK's exit from the EU and were adopted by the FSA and FSS on exit.

2.2. Section II: Identity, characterisation and condition of use

2.2.1. Characterisation of the active substance and the additive

The additive is a dry powder consisting of three active agents (*B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840; 8:5:3 ratio), with a guaranteed combined minimum concentration of 3.2 x 10^9 CFU/g additive. The additive's formulation is the same as the current authorisation and so the data detailing impurities, physico-chemical properties, and shelf life apply to the current assessment. The FEEDAP Panel concluded in 2020 that the active agents were fully characterised

as per the requirements of the FEEDAP guidance on the characterisation of microorganisms used as feed additives or production organisms (EFSA FEEDAP Panel, 2018b, EFSA, 2020).

For modification to the authorisation, the applicant presented further data to confirm the taxonomical identification and demonstrate their susceptibility to antibiotics. Taxonomical identification was achieved through bioinformatic analysis of whole genome sequencing data, confirming the identity of *B. subtilis* DSM 32324, *B. subtilis* DSM 32325, and *B. amylolyquefaciens* DSM 25840. Evaluation of the antimicrobial susceptibility of the three strains was performed by a broth dilution method using the list of antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2018b). All three strains were susceptible to the recommended antibiotics, returning minimum inhibitory concentrations below the cut-offs. Antimicrobial resistance (AMR) genes were also investigated through bioinformatic interrogation of the WGS data, identifying no hits that were considered a safety concern (EFSA, 2023).

The FSA and FSS agree with the conclusions reached for the characterisation of the additive and active agent. The studies were reviewed by EFSA in 2020, prior to the UK's exit from the EU; thus, this opinion is applicable to GB. These studies formed part of the assessment leading to the current authorisation of the additive in GB. The certificates of analysis were reviewed by the FSA and FSS and confirmed compliance with the specifications. The identity and the manufacturing process of the additive is not changed for the current application made to the FSA and FSS and as such has not been subject to further assessment. The characterisation of the feed additive is provided as per the existing authorisation and as assessed by EFSA.

2.2.2. Conditions of use

The additive is currently authorised for all poultry species for fattening and reared for laying/breeding at a minimum content of 1.6×10^9 CFU/kg complete feed and 5.4×10^8 CFU/L for water for drinking. The applicant is seeking modification to the proposed conditions of use of the additive, to allow the simultaneous use of the additive with approved coccidiostats monensin, salinomycin, narasin, nicarbazin+narasin and lasalocid.

The applicant requested the same conditions of use as EFSA evaluated in their latest opinion (EFSA, 2023). The FSA/FSS agree with the conditions of use proposed by the applicant.

2.2.3. Conclusions on Section II

The additive was fully characterised, and the identity of the active agents confirmed in the previous EFSA opinion (EFSA, 2020). The Panel reviewed the updated information provided for assessment, confirming taxonomical identification of the active agents and demonstrating susceptibility to the relevant antibiotics (EFSA, 2023).

The FSA/FSS agree with the conclusions reached on the data, which is supported by the guidance that is also applicable in GB.

2.3. Section III: Safety

2.3.1. Safety for the target animals, consumers and the environment

In its previous opinion, the Panel concluded that the active agents met the requirements for the QPS approach to assessment and were presumed safe for the target animal, consumers and the environment (EFSA, 2020). The applicant did not provide any new data to make the FEEDAP Panel reconsider previous conclusions. The Panel concluded that the proposed modification would not influence these previous conclusions.

The FSA and FSS agree with the conclusions reached on the data and the used qualified presumption of safety (QPS) approach, as this approach has been previously used in GB.

2.3.2. Safety for the user

In the previous EFSA opinion (EFSA, 2020), the Panel were unable to conclude on skin/eye irritation and skin sensitisation owing to the absence of data. For the modification to the authorisation the applicant provided an *in vitro* skin irritation potential study (OECD 439), and an *in vitro* eye irritation potential study (OECD 492), demonstrating the additive to not be an irritant to the eyes or skin (EFSA, 2023). No data were provided on skin sensitisation, however the FEEDAP noted that no validated assays are available for assessing the sensitisation potential of microorganisms.

EFSA considered GalliPro® Fit to be a non-irritant to eyes and skin but should be considered a respiratory sensitiser due to the proteinaceous nature of the active ingredients. No conclusions could be drawn on the potential of this additive to be a skin sensitiser as no data were provided.

The FSA and FSS agree with the conclusions reached for the safety of the user.

2.3.3. Conclusions on Section III: Safety

The FEEDAP Panel concluded that the proposed change to the authorisation would not affect the previous conclusions for safety for the target species, the consumer or the environment. The new data provided for assessment allowed conclusions that the additive is not a skin or eye irritant. However, the additive should be considered a respiratory sensitiser due to its proteinaceous nature. The skin sensitisation potential of the additive could not be concluded upon.

The FSA/FSS agree with the conclusions reached on the data, which is supported by the guidance that is also applicable in GB.

2.4. Compatibility with coccidiostats

The FEEDAP Panel concluded in the previous opinion that the strains composing the additive in its final form were compatible with dicluzaril, decoquinate and halofuginone, however they were unable to conclude on compatibility with monesin, salinomycin sodium, narasin, robenidine hydrochloride or maduramicin ammonium (EFSA, 2020). For the proposed modification the applicant provided an *in vivo* study to demonstrate the compatibility of the additive with several of these approved coccidiostats (Table 2).

Table 2. Effect of coccidiostats on the counts of caecal contents of birds fed with GalliPro® Fit

Treatment	Mean of the colony counts of *Bacillus*-like colonies (log CFU/g ± standard deviation) in broiler caecum samples		
	Number of samples	Non-heated samples	Heat treated samples
Negative control	17	2.2±0.5	2.2±0.3
GalliPro® Fit control	14/20	4.6±0.3 4.5±0.3*	4.6±0.3 4.5±0.3*
GalliPro® Fit + 125 mg Monensin/kg feed	17	4.6±0.3	4.6±0.2
GalliPro® Fit + 70 mg Narasin/kg feed	18	4.6±0.3	4.6±0.4
GalliPro® Fit + 70 mg Salinomycin/ kg feed	16	4.5±0.3	4.6±0.3
GalliPro® Fit + 50 mg Nicarbazin/kg feed + 50 mg narasin/kg feed	14	4.7±0.2	4.8±0.2
GalliPro® Fit + 6 mg lasalocid/kg feed	20	4.7±0.4	4.7±0.4

*Control used in lasalocid test

The data were reviewed by the Panel, who concluded that the data demonstrated that GalliPro® Fit is compatible for use with monensin, salinomycin, narasin, nicarbazin+narasin and lasalocid (EFSA, 2023). No data was provided to assess compatibility of the additive with robenidine hydrochloride and maduramicin ammonium.

The FSA and FSS agree with the conclusions reached on the data provided to demonstrate the compatibility of the additive with the coccidiostats monensin, salinomycin, narasin, nicarbazin+narasin and lasalocid.

3. Analytical methods evaluation

The FSA/FSS evaluated the EURL analytical method evaluation, noting it was carried out in 2019, when the UK was still part of the EU and would have participated of their approval. No concerns are raised at this stage for the validity of the methods for UK/GB use, and therefore, the FSA/FSS accept the EURL analytical method evaluation report (EURL, 2019). The FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The FEEDAP Panel concluded that the proposed modification to conditions of use of GalliPro® Fit would not influence the conclusions of the previous authorisation, and that the additive remains safe for the target animal, consumer and the environment. The *in vitro* studies provided to demonstrate user safety allowed a conclusion to be drawn that this additive is a non-irritant to the skin and eyes. The additive should be considered a potential respiratory sensitiser due to its proteinaceous nature. The skin sensitisation potential of the additive could not be determined.

The FEEDAP Panel concluded that the data provided demonstrated the compatibility of the additive with the approved coccidiostats: monensin, salinomycin, narasin, nicarbazin+narasin and lasalocid.

5. Caveats and uncertainties

No conclusion can be drawn on the skin sensitisation potential of the additive.

No data were provided to assess compatibility of the additive with robenidine hydrochloride and maduramicin ammonium. The compatibility of these coccidiostats were inconclusive in the previous EFSA opinion (EFSA, 2020) but were not assessed in the current EFSA opinion (EFSA, 2023).

6. FSA/FSS conclusions for GB risk analysis

The application has been assessed in line with the applicable guidance and is partially based on considerations of detailed proprietary information available to the Panel, which were also submitted to the FSA and FSS. The EFSA opinion identifies and characterises the hazards present from this proposed modification of use and there is sufficient information to enable an assessment of exposure, which is also relevant to GB. The risk characterisation is unchanged from the 2020 opinion for most areas, and appropriate evidence was submitted to support the requested modifications, including data to assess safety for the user. The conclusions of the EFSA opinion have been reviewed in detail by the FSA and FSS and are considered appropriate and consistent, including the caveats and uncertainties identified in the opinion which are applicable to GB. Sufficient evidence has been demonstrated to conclude without further questions or risk assessment.

Abbreviations

Abbreviation	Definition
AMR	Antimicrobial resistance
CFU	Colony-forming units
EC	European Commission
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FSA	Food Standards Agency
FSS	Food Standards Scotland
GB	Great Britain
MIC	Minimum inhibitory concentration
OECD	The Organisation for Economic Co-operation and Development
QPS	Qualified presumption of safety

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