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## Safety and efficacy of GalliPro<sup>®</sup> Fit (*Bacillus subtilis* DSM 32324, *Bacillus subtilis* DSM 32325 and *Bacillus amyloliquefaciens* DSM 25840) for all poultry species for fattening or reared for laying/breeding

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### Abstract

Following a request from the European Commission, the 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 GalliPro<sup>®</sup> Fit (*Bacillus subtilis* DSM 32324, *B. subtilis* DSM 32325 and *Bacillus amyloliquefaciens* DSM 25840) when used as a zootechnical additive in feed and water for drinking for all poultry species for fattening or reared for laying/breeding. The two bacterial species present in the additive are considered suitable for the qualified presumption of safety (QPS) approach to safety assessment. The identity of the active agents was established and the lack of toxigenic potential confirmed. The strains did not show resistance to relevant antibiotics. Therefore, the additive was presumed safe for the target species, consumers and the environment. In the absence of data, no conclusions could be drawn on the skin/eye irritancy or skin sensitisation of the additive. Due to the proteinaceous nature of the active agents, the additive was considered a respiratory sensitiser. The Panel concluded that GalliPro<sup>®</sup> Fit is compatible with diclazuril, decoquinate and halofuginone. However, the data provided did not allow to conclude on the compatibility of the additive with other coccidiostats. The Panel concluded that the additive has a potential to be efficacious in chickens for fattening at  $1.6 \times 10^9$  CFU/kg feed and at  $5.4 \times 10^8$  CFU/L water for drinking. The conclusion was extrapolated to all other poultry species for fattening or reared for laying/breeding. The results from the study in turkeys support this conclusion.

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**Keywords:** GalliPro<sup>®</sup> Fit, zootechnical additive, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, growing poultry species, safety, efficacy

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## 1. Introduction

### 1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003<sup>1</sup> 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 Chr. Hansen A/S<sup>2</sup> for authorisation of the product GalliPro® Fit (*Bacillus subtilis* DSM 32324, *B. subtilis* DSM 32325 and *Bacillus amyloliquefaciens* DSM 25840), when used as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and other minor growing poultry species (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 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 8 April 2019.

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® Fit (*B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840), when used under the proposed conditions of use (see Section 3.1.4).

### 1.2. Additional information

The product under assessment is based on viable spores of *B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840 and is not authorised as a feed additive in the European Union.

## 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<sup>3</sup> in support of the authorisation request for the use of GalliPro® Fit (*B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840) as an additive in feed and water for drinking.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active agents in animal feed. The Executive Summary of the EURL report can be found in Annex A.<sup>4</sup>

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of GalliPro® Fit (*B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840) is in line with the principles laid down in Regulation (EC) No 429/2008<sup>5</sup> and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed

<sup>1</sup> 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.

<sup>2</sup> Chr Hansen A/S, 10-12 Boege Allé, 2970 Hoersholm, Denmark.

<sup>3</sup> FEED dossier reference: FAD-2019-0009.

<sup>4</sup> The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2019-0009-galliprofit.pdf>

<sup>5</sup> 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.

additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019a).

### 3. Assessment

GalliPro® Fit (*B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840) is intended to be used as a zootechnical additive (functional group: gut flora stabiliser) in feed and water for drinking for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and other minor growing poultry species.

#### 3.1. Characterisation

##### 3.1.1. Characterisation of the active agents

The three strains were isolated in 2011 from faeces collected from healthy adult pigs in Germany.<sup>6</sup> All strains are deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen with the accession numbers DSM 32324 and DSM 32325 for the two *B. subtilis* strains and DSM 25840 for the *B. amyloliquefaciens* strain.<sup>7</sup>

None of the strains have been genetically modified.

Taxonomic identification of strains DSM 32324 and DSM 32325 as *B. subtilis* was established by bioinformatic analysis of the whole genome sequences (WGS). This was based on multi-locus sequence analysis (16S rRNA gene and partial *groEL*, *gyrA*, *polC*, *purH* and *rpoB* gene sequences) and average nucleotide identity (ANI) values of 98.2% (*B. subtilis* DSM 32324), 98.6% (*B. subtilis* DSM 32325) obtained for the comparison with genome sequences of the type strain.<sup>10</sup> Using the same methodology, the identity of DSM 25840 as *B. amyloliquefaciens* was also confirmed (ANI value of 99.5%).<sup>11</sup>

The toxigenic potential of each strain was assessed according to the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a).<sup>12</sup> No lysis of Vero cells was detected with any of the three strains. Therefore, *B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840 are considered to be non-toxicogenic.

The susceptibility of each of the three strains to the antibiotics recommended by the FEEDAP Guidance (EFSA FEEDAP Panel, 2018a) was tested by broth microdilution following the method of the Clinical and Laboratory Standards Institute (CLSI) and all of the minimum inhibitory concentration (MIC) values determined fell below the FEEDAP cut-off values.<sup>13</sup> The WGS of each active agent was interrogated for the presence of antimicrobial resistance genes (AMR)

No relevant hits were identified.

##### 3.1.2. Characterisation of the additive

<sup>6</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_2\_1\_2c.

<sup>7</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_2\_1\_2a.

<sup>10</sup> Technical dossier/Supplementary information August 2019/EFSA\_GPFit\_FAD-2019-0009\_08.2019/Annex\_Q1\_2 and Annex\_Q1\_3.

<sup>11</sup> Technical dossier/Supplementary information August 2019/EFSA\_GPFit\_FAD-2019-0009\_08.2019/Annex\_Q1\_1.

<sup>12</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_2\_2\_2a\_Cytotoxicity\_KG, Annex\_II\_2\_2\_2a\_Cytotoxicity\_QH and Annex\_II\_2\_2\_2a\_Cytotoxicity\_ZM16.

<sup>13</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_2\_2\_2b.

The carrier represents approximately 98% (w/w) of the final additive. The guaranteed minimum total concentration of viable spores of *Bacillus* spp. in the product is  $3.2 \times 10^9$  CFU/g additive ( $1.6 \times 10^9$  CFU *B. subtilis* DSM 32324/g;  $1.0 \times 10^9$  CFU *B. subtilis* DSM 32325/g and  $0.6 \times 10^9$  CFU *B. amyloliquefaciens* DSM 25840/g). Compliance was demonstrated in five batches of the additive assayed in duplicate by identifying colonies after culturing by specific quantitative polymerase chain reaction (qPCR) ( $1.7 \times 10^9$  CFU/g ( $1.5$ – $1.9 \times 10^9$  CFU/g) *B. subtilis* DSM 32324;  $1.5 \times 10^9$  CFU/g ( $1.1$ – $1.8 \times 10^9$  CFU/g) *B. subtilis* DSM 32325 and  $0.5 \times 10^9$  CFU/g ( $0.4$ – $0.7 \times 10^9$  CFU/g) *B. amyloliquefaciens* DSM 25840).<sup>18</sup>

Four batches of the additive were analysed for heavy metals and arsenic (lead, cadmium, mercury and arsenic were detected at mean concentrations of 0.31, 0.07, 0.003 and 0.26 mg/kg additive, respectively) and aflatoxin B1 (< LOQ) and they were in compliance with the maximum levels specified.<sup>19</sup> Analysis of microbial contamination in six batches indicated *Bacillus cereus*, coliforms and yeasts and moulds were <  $10^3$  CFU/g, *Escherichia coli* was < 10 CFU/g, while *Salmonella* spp. was absent in 25 g.<sup>20</sup> Chemical and microbiological analyses did not raise any concern.

The additive is a slightly hygroscopic, free-flowing powder with a white to yellowish colour. The particle size was measured in three batches using laser diffraction, particles below 100  $\mu\text{m}$  amounted up to 10.5%, particles below 10  $\mu\text{m}$  amounted up to 5.3%.<sup>21</sup> The dusting potential of the additive was measured in the same three batches following the Stauber-Heubach method and the mean value was  $5.25 \text{ g/m}^3$  ( $4.85$ – $6.05 \text{ g/m}^3$ ).<sup>22</sup> The bulk density and the tapped density measured in three batches gave a mean value of 1,500 and 1,700  $\text{kg/m}^3$ , respectively.<sup>23</sup>

### 3.1.3. Stability and homogeneity

The shelf-life of the additive was determined by monitoring three batches stored at 25°C, 30°C and 37°C for a period of 6 months, one batch at ambient temperature (25°C) for 12 months and three batches stored at 50°C for 3 months.<sup>24</sup> The results after 6 months showed a recovery between 96 and 105% compared to the initial counts at temperatures up to 37°C and a recovery of 103% at 25°C for 12 months. The results after 3 months at 50°C showed a recovery between 62 and 78% compared to the initial counts.

Three batches of the additive were individually mixed into a standard chicken mineral premixture (without choline chloride) at a concentration of  $2.7 \times 10^7$  CFU/g premixture and samples were stored for 6 months at 20–25°C.<sup>25</sup> The results after 6 months showed that the three batches had a recovery between 86 and 104% compared to the initial counts.

Stability in complete feed was investigated using three batches of the additive incorporated into a typical mash feed for chickens (corn and soybean) and into a pelleted feed of the same composition (pelleting conditions 95°C) at two concentrations of  $6.0 \times 10^8$  and  $1.2 \times 10^9$  CFU/kg feed.<sup>26</sup> Samples of the mash and pelleted feed were stored for up to 3 months at 20–25°C. Counts of total bacilli were made at the start and after 1 and 3 months. The results showed a recovery between 102–116% and 92–106% compared to the initial counts in mash and pelleted feed, respectively.

The stability studies of GalliPro® Fit during pelleting of poultry feed (pelleting temperature: 75, 85 and 95°C) showed a small loss of bacilli that was less than 0.5 Log difference.<sup>27</sup>

The stability of the additive (three batches) in water ( $3.2 \times 10^6$  CFU/mL water) was studied during storage at ambient temperature (25°C) for up to 48 h. The results showed that the number of bacilli after 24 and 48 h was 0.1 Log and 0.2 Log CFU/ml less than the time zero count, respectively.<sup>28</sup>

<sup>18</sup> Technical dossier/Supplementary information February 2020/EFSA\_GPFit\_FAD-2019-0009\_02.2020/Report\_colony\_qPCR\_GPFIT\_5\_batches\_02.2020.

<sup>19</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_1\_4\_1; LOQ for aflatoxin B1 was 0.64  $\mu\text{g/kg}$ .

<sup>20</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_1\_3b.

<sup>21</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_1\_5a.

<sup>22</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_1\_5b.

<sup>23</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_1\_5c.

<sup>24</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_4\_1a.

<sup>25</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_4\_1d.

<sup>26</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_4\_1c.

<sup>27</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_4\_1b.

<sup>28</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_4\_1e.



A total of 10 subsamples were taken from the pelleted feed and analysed for total bacilli counts. Based on the 10 samples, the coefficient of variation was 14%.<sup>29</sup>

### 3.1.4. Conditions of use

The product is proposed for use in feed and water for drinking for all poultry species for fattening or reared for laying/breeding at a minimum inclusion level of  $8.0 \times 10^8$  CFU/kg complete feedingstuffs and of  $2.7 \times 10^8$  CFU/L of water for drinking. The applicant requests for the simultaneous use of the additive with the following coccidiostats: decoquinat, diclazuril, halofuginone, monensin sodium, maduramicin ammonium, narasin, robenidine and salinomycin sodium.

## 3.2. Safety

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

The strains *B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840 belong to species considered to qualify for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007 and EFSA BIOHAZ Panel, 2020). This approach requires the identity of the strains to be conclusively established and evidence that the strains lack toxigenic potential and do not show acquired resistance to antibiotics of human and veterinary importance.

The FEEDAP Panel noted that the identity of the strains has been unambiguously established. Evidence was provided on the lack of toxigenic potential of the strains and on the absence of acquired antimicrobial resistance genes. Therefore, the strains do not raise safety concerns for the target species, consumers of products derived from animals fed the additive and the environment. Since the other components of the additive do not give rise to concerns, GalliPro® Fit is considered safe for the target species, consumer and the environment.

### 3.2.2. Safety for user

No studies, other than those relating to dust formation, were provided by the applicant on the safety of the additive for users. Therefore, no conclusions can be drawn on the skin/eye irritancy or skin sensitisation of the additive.

The high dusting potential (up to  $6.05 \text{ g/m}^3$ ) indicates that exposure by inhalation is likely to occur. Due to the proteinaceous nature of the active agents, the additive should be considered a respiratory sensitiser.

## 3.3. Efficacy

### 3.3.1. Efficacy for chickens for fattening

A total of five trials with chickens for fattening were submitted. One of the studies [REDACTED] was not considered further in the assessment [REDACTED]

[REDACTED] For the other four studies, the details on the study design are provided in Table 1 and the main results in Table 2. [REDACTED]

<sup>29</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_4\_2. [REDACTED]

[REDACTED]					
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

[REDACTED]

[REDACTED]

Based on the results, the Panel concludes that the additive has a potential to be efficacious as a zootechnical additive in chickens for fattening at the level of  $1.6 \times 10^9$  CFU/kg feed. The conclusion is extended to the use of the additive in water for drinking at the corresponding level of  $5.4 \times 10^8$  CFU/L.

### 3.3.2. Efficacy for turkeys for fattening

The applicant also provided a performance trial conducted in turkeys for fattening. [REDACTED]

[REDACTED]

[REDACTED]



[REDACTED]

3

[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

### 3.3.3. Compatibility with coccidiostats

The applicant provided *in vitro* studies to support the compatibility of *B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840 with diclazuril, decoquinat and halofuginone and *in vitro* and *in vivo* studies to support the compatibility of the three strains with monensin sodium, salinomycin sodium, narasin, robenidine hydrochloride and maduramicin ammonium.

MIC values against the individual active agents were assessed using the broth microdilution method in aerobic conditions *in vitro*.<sup>36</sup> The MIC values for diclazuril (> 19.2 mg/L), decoquinat (> 640 mg/L) and halofuginone (> 48 mg/L) were greater than four times their maximum authorised level (1.2, 40 and 3 mg/kg, respectively).<sup>37</sup> The MIC values<sup>38</sup> for monensin sodium (0.98 mg/L), salinomycin sodium (1.09 mg/L), narasin (1.09 mg/L), robenidine hydrochloride (0.545 mg/L) and maduramicin ammonium (3 mg/L) were below four times their maximum authorised dose (125, 70, 70, 36 and 6 mg/kg, respectively).

The results indicate that GalliPro® Fit is compatible with diclazuril, decoquinat and halofuginone; however, as the MIC values for monensin sodium, salinomycin sodium, narasin, robenidine hydrochloride and maduramicin ammonium were less than four times the maximum authorised level for these coccidiostats, *in vivo* studies were submitted.

Three *in vivo* studies were performed on each of the strains separately as described below.

In each trial,

[REDACTED]

<sup>36</sup> Technical dossier/Section II/Annexes Sect.II/Annex\_II\_4\_4a.

<sup>37</sup> Maximum authorised levels: diclazuril 1 mg/kg (chickens for fattening, guinea fowls, chickens reared for laying and turkey for fattening); decoquinat 40 mg/kg; (chickens for fattening); halofuginone 3 mg/kg (chickens for fattening and turkeys); monensin sodium 125 mg/kg (chickens for fattening and chickens reared for laying) and 100 mg/kg (turkeys); salinomycin sodium 70 mg/kg (chickens for fattening) and 50 mg/kg (chickens reared for laying); narasin 70 mg/kg (chickens for fattening); robenidine hydrochloride 36 mg/kg (chickens for fattening and turkeys); maduramicin ammonium 6 mg/kg (chickens for fattening) and 5 mg/kg (turkeys).

<sup>38</sup> Data shown is for the most sensitive strain, *B. amyloliquefaciens* DSM 25840.

[REDACTED]

[REDACTED]

The results are given in Table 4.

[REDACTED]

[REDACTED]	[REDACTED]					
	[REDACTED]		[REDACTED]		[REDACTED]	
	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	■	■	■	■	■	■
[REDACTED]	■	■	■	■	■	■
[REDACTED]	■	■	■	■	■	■
[REDACTED]	■	■	■	■	■	■
[REDACTED]	■	■	■	■	■	■
[REDACTED]	■	■	■	■	■	■
[REDACTED]	■	■	■	■	■	■

[REDACTED]

the data provided do not allow to conclude on the compatibility of *B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840 with monensin sodium, salinomycin sodium, narasin, robenidine hydrochloride and maduramicin ammonium.

**3.3.3.1. Conclusions on efficacy**

Based on the results of the trials conducted in chickens for fattening, the Panel concludes that the additive has a potential to be efficacious as a zootechnical additive in chickens for fattening at  $1.6 \times 10^9$  CFU/kg feed and at  $5.4 \times 10^8$  CFU/L water for drinking. This conclusion can be extended to chickens reared for laying.

Since the effects of the additive can be reasonably assumed to be the same between poultry species, the Panel considers that the conclusions from the efficacy studies in chickens for fattening can be used to conclude on turkeys for fattening, turkeys reared for breeding and other minor growing poultry species. The results from the study in turkeys support this conclusion.

Considering the data submitted, the FEEDAP Panel concludes that GalliPro® Fit is compatible with diclazuril, decoquinate and halofuginone. The data provided do not allow to conclude on the compatibility of the additive with monensin sodium, salinomycin sodium, narasin, robenidine hydrochloride and maduramicin ammonium.

### 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 Regulation<sup>40</sup> and Good Manufacturing Practice.

## 4. Conclusions

The three active agents included in the additive (*B. subtilis* DSM 32324, *B. subtilis* DSM 32325 and *B. amyloliquefaciens* DSM 25840) meet the requirements of the QPS approach to safety assessment and are presumed safe for the target animals, consumers of products derived from animals fed the additive and the environment. Since the other components of the additive do not give rise to concerns, GalliPro® Fit is also considered safe for the target species, consumer and the environment.

In the absence of data, no conclusions on the skin/eye irritancy or skin sensitisation of the additive can be made. Due to the proteinaceous nature of the active agents, the additive should be considered a respiratory sensitiser.

GalliPro® Fit has a potential to be efficacious as a zootechnical additive in chickens for fattening at  $1.6 \times 10^9$  CFU/kg feed and at  $5.4 \times 10^8$  CFU/L water for drinking. This conclusion is extended to chickens reared for laying or breeding and is extrapolated to turkeys for fattening, turkeys reared for breeding and minor growing poultry species. The results from the study in turkeys support this conclusion.

The FEEDAP Panel concludes that GalliPro® Fit is compatible with diclazuril, decoquinat and halofuginone. The Panel cannot conclude on the compatibility of the additive with monensin sodium, salinomycin sodium, narasin, robenidine hydrochloride and maduramicin ammonium.

## 5. Documentation as provided to EFSA/Chronology

Date	Event
12/02/2019	Dossier received by EFSA. GalliPro® Fit ( <i>Bacillus subtilis</i> DSM 32324, <i>Bacillus subtilis</i> DSM 32325 and <i>Bacillus amyloliquefaciens</i> DSM 25840) for all poultry species for fattening or reared for laying/breeding. Submitted by Chr. Hansen A/S.
25/02/2019	Reception mandate from the European Commission
08/04/2019	Application validated by EFSA – Start of the scientific assessment
05/06/2019	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; user safety; efficacy</i>
10/07/2019	Comments received from Member States
22/07/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
02/08/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
21/10/2019	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.</i>
26/02/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
20/03/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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<sup>40</sup> 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.

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## Abbreviations

AMR	antimicrobial resistance genes
ANI	Average nucleotide identity
CFU	Colony-forming units
EURL	European Union Reference Laboratory
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
qPCR	Quantitative Polymerase Chain Reaction
QPS	Qualified presumption of safety
MIC	Minimum inhibitory concentration
WGS	Whole genome sequences

## Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for GalliPro® Fit

In the current application authorisation is sought under Article 4(1) for a preparation of *Bacillus subtilis* DSM 32324, *Bacillus subtilis* DSM32325 and *Bacillus amyloliquefaciens* DSM25840 (*GalliPro*® *Fit*) under the category/functional group 4(b) 'zootechnical additives'/gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and other growing poultry species.

According to the Applicant, the *feed additive* contains as *active substances* viable spores of non-genetically modified strains *Bacillus subtilis* DSM 32324, *Bacillus subtilis* DSM 32325 and *Bacillus amyloliquefaciens* DSM 25840. The *feed additive* is to be marketed as a powder containing a minimum content of total *active substances* of  $3.2 \times 10^9$  Colony Forming Unit (CFU)/g. The *feed additive* is intended to be used directly in *feedingstuffs* or through *premixtures* at a minimum dose of  $8 \times 10^8$  CFU/kg complete *feedingstuffs* and in drinking *water* at a minimum dose of  $2.7 \times 10^8$  CFU/L.

For the identification of *Bacillus subtilis* DSM 32324, *Bacillus subtilis* DSM32325 and *Bacillus amyloliquefaciens* DSM25840, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for genetic identification of bacterial strains.

For the enumeration of the overall *Bacillus* spp. (DSM 32324, DSM 32325 and DSM 25840) in the *feed additive*, *premixtures*, *feedingstuffs* and drinking *water* the Applicant submitted the ring-trial validated spread plate CEN method EN 15784. Based on the performance characteristics available, the EURL recommends this method for official control for the enumeration of the overall *Bacillus* spp. (DSM 32324, DSM 32325 and DSM 25840) in the *feed additive*, *premixtures*, *feedingstuffs* and *water*.

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), as last amended by Regulation (EU) 2015/1761) is not considered necessary.