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Modification of the conditions of the authorisation of BioPlus[®] 2B (*Bacillus licheniformis* DSM 5749 and *Bacillus subtilis* DSM 5750) for turkeys for fattening

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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 modification of the terms of the authorisation of BioPlus[®] 2B (*Bacillus licheniformis* DSM 5749 and *Bacillus subtilis* DSM 5750) in feeds for turkeys for fattening to allow the simultaneous use with a battery of permitted coccidiostats (diclazuril, halofuginone, monensin sodium, robenidine hydrochloride, maduramicin ammonium, lasalocid sodium) and with the preservative formic acid. The proposed modification in the conditions of the authorisation would not modify the conclusions previously drawn regarding the safety of the additive. The additive is safe for the target species, consumers and the environment. The additive should be considered a potential respiratory sensitiser, but the Panel could not conclude on the irritancy of the additive to skin and eyes or its dermal sensitisation. Conclusions previously drawn by the FEEDAP Panel on the compatibility of the additive for chickens for fattening apply to the current application provided that the maximum authorised concentration of the coccidiostats semduramycin, maduramicin ammonium, lasalocid sodium and the preservative formic acid for turkeys for fattening (when maximum authorised concentrations exist), are equal or lower than those for chickens for fattening. Considering the data submitted, the FEEDAP Panel concludes that BioPlus[®] 2B (*B. licheniformis* DSM 5749 and *B. subtilis* DSM 5750) is compatible with diclazuril and monensin sodium. Based on the data provided, no conclusion can be drawn for robenidine hydrochloride. In the absence of data, no conclusion can be drawn for halofuginone.

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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 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States.

The European Commission received a request from Chr. Hansen A/S² for modifications of the terms of authorisation of the product BioPlus[®] 2B (*Bacillus licheniformis* DSM 5749 and *Bacillus subtilis* DSM 5750), when used as a feed additive for turkeys for fattening (category: zootechnical additives; functional group: gut flora stabilisers).

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 13(3) (modification of the authorisation of a 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 09 October 2018.

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 BioPlus[®] 2B (*Bacillus licheniformis* DSM 5749 and *Bacillus subtilis* DSM 5750), when used under the proposed conditions of use (Section 3.1).

1.2. Additional information

The additive BioPlus[®] 2B is a preparation of *B. licheniformis* (DSM 5749) and *B. subtilis* (DSM 5750). EFSA has issued several opinions on the compatibility of BioPlus[®] 2B with coccidiostats (EFSA, 2003, 2006, 2007b, 2008; EFSA FEEDAP Panel, 2005, 2011, 2016) and one opinion on the use of the additive with sows (EFSA FEEDAP Panel, 2011b).

The additive is currently authorised for use in feed for pigs for fattening and piglets, sows, turkeys for fattening and calves.³ With the current application, the applicant requests to include the compatibility with coccidiostats and formic acid to the current authorisation for turkeys.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁴ in support of the authorisation request for the use of BioPlus[®] 2B (*B. licheniformis* DSM 5749 and *B. subtilis* DSM 5750) as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies to deliver the present output.

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.⁵

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, 2970, Hoersholm, Denmark.

³ Commission Implementing Regulation (EU) 2017/447 of 14 March 2017 concerning the authorisation of the preparation of *Bacillus subtilis* (DSM 5750) and *Bacillus licheniformis* (DSM 5749) as a feed additive for sows, weaned piglets, pigs for fattening, calves for rearing and turkeys for fattening and amending Regulations (EC) No 1453/2004, (EC) No 2148/2004 and (EC) No 600/2005 (holder of authorisation Chr. Hansen A/S) OJ L 69, 15.3.2017, p. 18–22. http://data.europa.eu/eli/reg_impl/2017/447/oj

⁴ FEED dossier reference: FAD-2018-0061.

⁵ The report linked to the previous dossier (related to EFSA-Q-2008-381) is available on the EURL website: <https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2009-0023.pdf>

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of BioPlus[®] 2B (*B. licheniformis* DSM 5749 and *B. subtilis* DSM 5750) is in line with the principles laid down in Regulation (EC) No 429/2008⁶ and the relevant guidance documents: Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018).

3. Assessment

The additive BioPlus 2B[®] is a preparation of viable spores of *B. licheniformis* DSM 5749 and *B. subtilis* DSM 5750 intended for use in feed for turkeys for fattening (category: zootechnical additives; functional group: gut flora stabilisers). The product has already been authorised as a zootechnical additive (functional group: gut flora stabilisers) for use in diets and water for drinking for several animal species, including turkeys for fattening at the dose of 1.3×10^9 colony forming units (CFU)/kg complete feedingstuffs and 6.5×10^8 CFU/L water.⁷ The applicant is requesting the modification of the terms of that authorisation to allow the simultaneous use of BioPlus[®] 2B in feeds for turkeys for fattening with the permitted coccidiostats diclazuril, halofuginone, monensin sodium, robenidine hydrochloride, maduramicin ammonium and lasalocid sodium and with formic acid.

3.1. Characterisation and conditions of use

The additive BioPlus[®] 2B is based on the spores of two active agents, *B. licheniformis* DSM 5749 and *B. subtilis* DSM 5750 in equal ratio at the concentration of at least 1.6×10^{10} CFU/g each on a carrier of limestone/calcium carbonate (92.9%) and 1.1% Kieselgur (E 551c) as an anticaking agent. The identity of the two active agents, their lack of toxigenic potential and their susceptibility to the relevant antibiotics were established in a previous opinion (EFSA FEEDAP Panel, 2016) in which the additive was properly characterised. No new information has been submitted with regard to the identity and characterisation of the additive and the Panel considers that the information previously assessed applies to the current assessment.

BioPlus 2B[®] is authorised for use in feedingstuffs and water for drinking for turkeys for fattening, at the minimum recommended level of 1.3×10^9 CFU/kg feed and 6.5×10^8 CFU/L water.

The applicant requests the modification of the current authorisation by adding the simultaneous use of the additive in compound feed for turkeys for fattening containing (i) the authorised coccidiostats: diclazuril, halofuginone, monensin sodium, robenidine hydrochloride, maduramicin ammonium, lasalocid sodium and (ii) the preservative formic acid.

3.2. Safety

The bacterial species *B. licheniformis* and *B. subtilis* are considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007a; EFSA BIOHAZ Panel, 2017). This approach requires the identity of the strains to be conclusively established and the demonstration that the strains are not toxigenic and susceptible to the relevant antibiotics. The identity of the strains and their lack of toxigenic potential and their susceptibility to all antibiotics tested was established in a previous opinion (EFSA FEEDAP Panel, 2016). In that opinion, the FEEDAP Panel concluded that the additive was safe for the target species, consumers and the environment. The additive should be considered a potential respiratory sensitiser, but the Panel could not conclude on the irritancy of the additive to skin and eyes or its dermal sensitisation. The Panel considers that the proposed modifications to the terms of the authorisation of the additive will not introduce safety concerns not already considered in the previous assessments.

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 Commission Implementing Regulation (EU) 2017/447 of 14 March 2017 concerning the authorisation of the preparation of *Bacillus subtilis* (DSM 5750) and *Bacillus licheniformis* (DSM 5749) as a feed additive for sows, weaned piglets, pigs for fattening, calves for rearing and turkeys for fattening and amending Regulations (EC) No 1453/2004, (EC) No 2148/2004 and (EC) No 600/2005 (holder of authorisation Chr. Hansen A/S).

3.3. Efficacy

3.3.1. Compatibility with coccidiostats

In previous opinions on the use of BioPlus[®] 2B in feed for chickens for fattening, the compatibility of *B. licheniformis* DSM 5749 and *B. subtilis* DSM 5750 with maduramicin ammonium (EFSA, 2006), semduramycin and formic acid (EFSA FEEDAP Panel, 2011) and lasalocid sodium (EFSA, 2008) at the highest authorised levels for chickens for fattening was established. Data on the compatibility of one of the strains (*B. licheniformis* DSM 5749) with monensin sodium was not available, while the compatibility of both strains with diclazuril, halofuginone or robenidine hydrochloride was not established (EFSA FEEDAP Panel, 2010).

During the course of the assessment, the applicant informed EFSA that the use of halofuginone was not relevant in the current application and did not provide data on the compatibility with halofuginone.

In the current application, the applicant provided *in vitro* studies to support the compatibility of the active agents of Bioplus 2B[®] with diclazuril and robenidine hydrochloride and *in vitro* and *in vivo* studies with monensin sodium. The *in vitro* study with monensin was not further assessed as no information was provided on the minimum inhibitory concentrations (MIC)

The MIC for diclazuril and robenidine hydrochloride were assessed [REDACTED]

The results indicate that Bioplus 2B[®] is compatible with diclazuril; [REDACTED] for robenidine hydrochlorides [REDACTED] an *in vivo* study would be required to support its compatibility. However such a study was not submitted.

One *in vitro* study and two *in vivo* studies were submitted to support the compatibility of the two microbial strains with monensin sodium. However, the *in vitro* study did not report the MIC values and therefore was not considered. Similarly, one of the *in vivo* studies was not considered [REDACTED] The remaining *in vivo* study is described below.

[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]

Conclusions on the compatibility previously drawn by the FEEDAP Panel for chickens for fattening apply to the current application provided that the maximum authorised concentration of the coccidiostats semduramycin, maduramicin ammonium, lasalocid sodium and the preservative formic acid for turkeys for fattening (when maximum authorised concentrations exist), are equal or lower than those for chickens for fattening.

Considering the data submitted, the FEEDAP Panel concludes that BioPlus® 2B (*B. licheniformis* DSM 5749 and *B. subtilis* DSM 5750) is compatible with diclazuril and monensin sodium. Based on the data provided, no conclusion can be drawn for robenidine hydrochloride. In the absence of data, no conclusion can be drawn for halofuginone.

3.3.2. 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 Regulation¹³ and Good Manufacturing Practice.

4. Conclusions

The proposed modification in the conditions of the authorisation would not modify the conclusions previously drawn regarding the safety of the additive. The additive is safe for the target species, consumers and the environment. The additive should be considered a potential respiratory sensitiser, but the Panel could not conclude on the irritancy of the additive to skin and eyes or its dermal sensitisation.

Conclusions previously drawn by the FEEDAP Panel on the compatibility of the additive for chickens for fattening apply to the current application provided that the maximum authorised concentration of the coccidiostats semduramycin, maduramicin ammonium, lasalocid sodium and the preservative formic acid for turkeys for fattening (when maximum authorised concentrations exist), are equal or lower than those for chickens for fattening.

Considering the data submitted, the FEEDAP Panel concludes that BioPlus® 2B (*B. licheniformis* DSM 5749 and *B. subtilis* DSM 5750) is compatible with diclazuril and monensin sodium. Based on the data provided, no conclusion can be drawn for robenidine hydrochloride. In the absence of data, no conclusion can be drawn for halofuginone.

Chronology

Date	Event
04/05/2017	Dossier received by EFSA. BioPlus® 2B (<i>Bacillus licheniformis</i> DSM 5749 and <i>Bacillus subtilis</i> DSM 5750) for turkeys for fattening. Chr. Hansen A/S.
13/08/2018	Dossier received by EFSA
28/08/2018	Reception mandate from the European Commission
09/10/2018	Application validated by EFSA – Start of the scientific assessment
06/10/2018	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: efficacy</i>
09/01/2019	Comments received from Member States
01/03/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
15/05/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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- EFSA (European Food Safety Authority), 2007a. 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(12):587, 16 pp. <https://doi.org/10.2903/j.efsa.2007.587>

¹³ 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

CD	Commission Decision
CFU	colony forming unit
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
MIC	minimum inhibitory concentration
QPS	Qualified Presumption of Safety