REGULATED PRODUCTS SAFETY ASSESSMENT

# Safety Assessment on the Safety and Efficacy of an Additive Containing *Bacillus Licheniformis* DSM5749 and *Bacillus Subtilis* DSM5750 (BioPlus® 2B) for Use in Piglets, Calves for Fattening and Other Growing ruminants. (RP1154)

Food Standards Agency[1], Food Standards Scotland[2]

[1] Regulated Products Risk Assessment, Food Standards Agency, UK, [2] Risk Assessment, Food Standards Scotland, UK

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FSA Research and Evidence

An application was submitted to the Food Standards Agency in June 2021 from Chr. Hansen A/S ('the applicant') for the new use of an additive (BioPlus® 2B) containing *Bacillus licheniformis* DSM5749 and *Bacillus subtilis* DSM5750, under the category of 'zootechnical additives' and functional group 'gut flora stabilisers'. The additive is proposed to be used at an inclusion rate of $1.3 \times 10^9$ CFU/kg of complete feed (12% moisture) and $6.5 \times 10^8$ CFU/L of drinking water for calves for fattening, other growing ruminants at the corresponding developmental stage, and suckling piglets during the period in which solid feed is given.

To support the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in evaluating the dossier, the Advisory Committee on Animal Feedingstuffs (ACAF) were asked to review the dossier and the supplementary information from the applicant.

The FSA/FSS concluded, based on the ACAF's advice, that the additive was correctly characterised and both microorganisms were confirmed to be QPS. No concerns were raised for Section II of the dossier.

The additive can be considered safe for the target species, consumers and the environment. The active substances are presumed to be respiratory sensitisers. In the absence of data, the additive should be considered a potential skin and eye irritant, and a skin sensitiser. It is recommended that methods are adopted to minimise worker exposure to the additive.

BioPlus® 2B, when used at the proposed doses of $1.3 \times 10^9$ CFU/kg of complete feed (12% moisture) and $6.5 \times 10^8$ CFU/L of drinking water, has the potential to be efficacious for calves for fattening and other growing

ruminants at the same developmental stage, as well as suckling piglets during the period in which solid feed is given. For suckling piglets, it is recommended to feed the additive to lactating sows simultaneously.

The views of ACAF have been taken into account in the safety assessment which represents the opinion of the FSA and FSS.

This is a joint FSA and FSS publication.

# 1. Introduction

The FSA and FSS have undertaken an assessment for a feed additive containing *Bacillus licheniformis* (also known as *Bacillus paralicheniformis*) DSM5749 and *Bacillus subtilis* DSM5750 (BioPlus® 2B, Chr. Hansen A/S, 10-12 Boege Allé, 2970, Hoersholm, Denmark) under assimilated Regulation (EC) No 1831/2003 (EC, 2003) in the category of 'zootechnical additives' and functional group 'gut flora stabilisers', for its use in calves for fattening, other growing ruminants at the corresponding developmental stage, and suckling piglets during the period in which solid feed is given.

To support the safety assessment by FSA and FSS, the ACAF provided advice to the FSA and FSS outlined in this document.

In line with Article 8 of 1831/2003, the assessment has considered and concluded that 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 EFSA for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

The dossier was evaluated by ACAF at their July 2023 meeting, after which a request for information was communicated to the applicant. The response to this request and subsequent requests was reviewed by the ACAF at their July 2024 meeting.

The views of ACAF have been taken into account in this safety assessment which represents the opinion of the FSA/FSS.

Table 1. Table showing products included in this assessment

| Title | Product type | Intended use/s | Intended species or categories of animals |
|---|---|---|---|
| *Bacillus licheniformis* DSM5749 and *Bacillus subtilis* DSM5750 | Feed additive | Zootechnical additive | Calves for fattening, other growing ruminants at the corresponding developmental stage, and suckling piglets during the period in which solid feed is given |

# 2. Assessment

## 2.1. Section II: Identity, characterisation and conditions of use

The additive is a preparation of non-GMO *Bacillus licheniformis* DSM5749 and *Bacillus subtilis* DSM5750, at a ratio of 1:1, and a minimum concentration of 3.2 x $10^{10}$ CFU/g of both microorganisms combined. The applicant provided data from several batches supporting the values outlined below (Table 2):

Table 2. Identity table for BioPlus 2B

| | |
|---|---|
| **Composition** | |
| *B. licheniformis* DSM5749 | 2.1 x $10^{10}$ CFU/g - (3%) |
| *B. subtilis* DSM5750 | 2.08 x $10^{10}$ CFU/g - (3%) |
| Calcium carbonate (carrier) | 92.9 % |
| Kieselgur (anti-caking agent) | 1.1 % |
| **Appearance** | |
| White to yellowish powder | |
| **Chemical-physical specifications** | |
| Dusting potential (average) | 6.26 g/$m^3$ |
| Particle-size distribution | > 100 µm – 71.9 %<br>< 100 µm – 28.1 %<br>< 50 µm – 13.5 %<br>< 10 µm – 4.8 %<br>< 4 µm – 0.3 %<br>< 1 µm – 0 % |
| Bulk density (average) | 1.3 g/ml |
| **Impurities** | |
| Arsenic | < 0.928 mg/kg |
| Lead | < 0.976 mg/kg |
| Cadmium | < 0.148 mg/kg |
| Mercury | < 0.067 mg/kg |
| Enterobacteriaceae | < 10 CFU/g |
| Yeasts and moulds | < 50 CFU/g |
| *Salmonella spp.* | Absent in 25 g |
| *Bacillus cereus* | < 10 CFU/g |
| Aflatoxin B1 | < 0.46 µg/kg |

Requests were made to the applicant to provide updated data on composition and impurities, as well as whole genome sequence (WGS) data analysis for the identification and characterisation of the bacterial strains that comprise the active substances of the additive. The Committee concluded that the WGS presented in the request for information confirmed the identity of *B. licheniformis* DSM5749 and *B. subtilis* DSM5750 as being QPS microorganisms. The additive showed stability in water, premixtures and feed. Stability was shown up to 95$^{o}$C after 10 minutes of exposure through pelleting. Homogeneity was also demonstrated. A shelf-life of 2 years was proposed and demonstrated for the additive. The proposed conditions of use of the additive are described in Table 3:

Table 3. Conditions of use of BioPlus 2B proposed by the applicant

| Conditions of use proposed by the applicant | | | | |
|---|---|---|---|---|
| **Species or category of animal** | **Max Age** | **Min. content in feed** | **Min. content in water** | **Withdrawal period** |
| Piglets (suckling – period in which solid feed is given) | Up to 35 kg | 1.3 x $10^9$ CFU/kg of complete feed | 6.5 x $10^8$ CFU/L of drinking water | -- |
| Calves for fattening | -- | 1.3 x $10^9$ CFU/kg of complete feed | 6.5 x $10^8$ CFU/L of drinking water | -- |
| Other growing ruminants | -- | 1.3 x $10^9$ CFU/kg of complete feed | 6.5 x $10^8$ CFU/L of drinking water | -- |
| **Other provisions and additional requirements for the labelling** | | | | |
| Specific conditions or restrictions for use | Store at ambient temperatures (20-25˚C) in original, closed packaging. Can be used in feed containing formic acid and the approved coccidiostats diclazuril, lasalocid sodium, monensin sodium, maduramicin ammonium, semduramicin sodium. | | | |
| Specific conditions or restrictions for handling | For user safety: recommended to use a facemask (ex. 3M 8835), gloves and goggles to reduce contact with dust. | | | |
| Post market monitoring | Chr. Hansen A/S will conduct post-marketing monitoring in compliance with EU law on feed hygiene, namely by use of HACCP and traceability systems, and formal monitoring of customer feedback through product or service complaints. | | | |
| Specific conditions for use in complementary feedingstuffs | Content of *Bacillus* spores in final feeds should be min. 1.3 x$10^9$ CFU/kg | | | |

## 2.1.1. Conclusions on Section II: Identity, characterisation and conditions of use

The ACAF concluded the additive was correctly characterised. No concerns were raised for Section II of the dossier.

# 2.2. Section III: Safety

*Bacillus licheniformis* DSM5749 and *Bacillus subtilis* DSM5750 were included in the Qualified Presumption of Safety (QPS) list in 2023. The species *B. licheniformis* and *B. subtilis* were included in the original QPS list in 2007, at a time when the UK was part of the EU. The ACAF raised no concern over the use of the QPS approach for the presumption of safety of these two strains.

## 2.2.1. Safety for the target species and consumers

No evaluation of the safety for the target species and consumers was required due to the QPS status confirmation of both *Bacillus licheniformis* DSM5749 and *Bacillus subtilis* DSM5750. The additive is presumed safe for the target species and consumers.

## 2.2.2. Safety for the user/worker

No new studies to evaluate the safety for the user were presented in this application. As the active substances are microorganisms, these are presumed to be respiratory sensitisers. In the absence of data, the additive was concluded to be a potential skin and eye irritant, as well as a skin sensitiser. Given the high dusting potential of the final formulation, the use of protective equipment in well-ventilated areas is recommended to minimise exposure.

## 2.2.3. Safety for the environment

As both microorganisms are QPS, the additive is presumed to be safe for the environment. The other components of the additive do not raise cause for concern from an environmental safety standpoint. No further testing was carried out.

## 2.2.4. Conclusions on safety

The ACAF concluded that the additive is safe for the target species, consumers and the environment, given the QPS status of the microorganisms that act as active substances of the additive. The ACAF raised no concern over the use of the QPS approach for the presumption of safety of these two bacterial strains.

The active substances are presumed to be respiratory sensitisers. In the absence of data, the additive should be considered a potential skin and eye irritant, and a skin sensitiser. It is recommended that methods are adopted to minimise worker exposure to the additive.

# 2.3. Section IV: Efficacy

## 2.3.1. Efficacy in calves for fattening and other growing ruminants

The applicant presented data from three studies previously evaluated by EFSA in their 2016 opinion (FEEDAP, 2016), where it was concluded that the additive has the potential to improve performance of calves for rearing. The applicant proposed to extrapolate the data to calves for fattening and other growing ruminants at the same developmental stage. The committee noted the disparity in stages of development as defined in legislation and guidance texts, and considered the validity of the shorter trials presented (56 days). It was concluded that the physiological status of calves would be very similar regardless of their purpose being for fattening or rearing, therefore the extrapolation of conclusions was accepted. It was concluded that the additive has the potential to be efficacious in calves for fattening and other growing ruminants at the same developmental stage.

## 2.3.2. Efficacy in piglets

The applicant presented data from three studies previously evaluated by EFSA in their 2016 opinion (FEEDAP, 2016), where it was concluded that the additive has the potential to improve performance of weaned piglets. After a request for further information, the applicant clarified that they proposed to extrapolate these conclusions to suckling piglets to the period in which solid feed is given, without the requirement to give the additive to lactating sows simultaneously. The Committee evaluated the proposal and concluded that the studies presented showed the additive has the potential to be efficacious for weaned piglets, and that these conclusions can be extrapolated to suckling piglets for the period in which solid feed is given. Members agreed with the current EU authorisation suggestion that the simultaneous feeding of the additive to sows should not be a requirement, but still remain as a recommendation.

## 2.3.3. Conclusions on efficacy

The ACAF concluded that BioPlus 2B has the potential to be efficacious for calves for fattening and other growing ruminants at the same developmental stage, as well as suckling piglets during the period in which solid feed is given. For suckling piglets, it is recommended to feed the additive to lactating sows simultaneously.

# 3. Analytical methods evaluation

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the European Union Reference Laboratory (EURL) for Feed Additives on the Method(s) of the Analysis for BioPlus® 2B (EURL, 2010). Reference is made to the Community Reference Laboratory (CRL), which is the predecessor body to the EURL:

> "Furthermore, the applicant suggested a minimum content of BioPlus® 2B ranging from 11 to 270 g (depending on the species) per 1000 animals in drinking water. For the enumeration of the sum of the two strains of the active agents in feed additive, premixtures, feedingstuffs and water the applicant proposes the ring trial validated spread plate method (EN 15784) using tryptone soya agar. The performance characteristics of the CEN method reported after logarithmic transformation of measured CFU values are:
>
> - a standard deviation for repeatability (sr) ranging from 0.07 to 0.09 $_{log}$10 CFU/g
>
> - a standard deviation for reproducibility (sR) ranging from of 0.32 to 0.35 $_{log}$10 CFU/g, and
>
> - a limit of detection (LOD) of $10^5$ CFU/kg of feedingstuffs.
>
> Based on these performance characteristics, the CRL recommends for official control the ring trial validated method EN 15784 for the determination of *Bacillus subtilis* DSM 5750 and *Bacillus licheniformis* DSM 5749 in feed additive, premixtures, feedingstuffs and water.
>
> Molecular methods were used by the Applicant for identification of the active agent. The CRL recommends for official control, Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification."

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical methods proposed as appropriate for official controls for this feed additive. The analytical methods were approved in 2010, at the time in which the UK was still an EU member.

# 4. Conclusions

The ACAF concluded the additive was correctly characterised and both microorganisms were confirmed to be QPS. No further concerns were raised for Section II of the dossier.

The ACAF concluded that the proposed level of inclusion in feed is safe for the target species, consumers and the environment.

The active substances are presumed to be respiratory sensitisers. In the absence of data, the additive should be considered a potential skin and eye irritant, and a skin sensitiser. It is recommended that methods are adopted to minimise worker exposure to the additive.

The ACAF concluded that BioPlus® 2B, when used at the proposed doses of 1.3 x $10^9$ CFU/kg of complete feed (12% moisture) and 6.5 x $10^8$ CFU/L of drinking water, has the potential to be efficacious for calves for fattening and other growing ruminants at the same developmental stage, as well as suckling piglets during the period in which solid feed is given. For suckling piglets, it is recommended to feed the additive to lactating sows simultaneously.

# Abbreviations

| Acronym | Definition |
|---|---|
| ACAF | Advisory Committee on Animal Feedingstuffs |
| CEN | Comité Européen de Normalisation |
| CFU | Colony forming units |
| CRL | Community Reference Laboratory |
| EC | European Commission |
| EFSA | European Food Safety Authority |
| EURL | European Union Reference Laboratory |
| FSA | Food Standards Agency |
| FSS | Food Standards Scotland |
| LOD | Limit of detection |
| PFGE | Pulsed field gel electrophoresis |
| QPS | Qualified presumption of safety |
| WGS | Whole Genome Sequence |

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