REGULATED PRODUCTS SAFETY ASSESSMENT

Assessment on Safety and Efficacy of the Feed Additive Consisting of *Bacillus Velezensis* CECT 5940 (Formerly Bacillus Amyloliquefaciens) for Turkeys for Fattening, Turkeys Reared for Breeding, Minor Poultry Species for Fattening and Reared for Laying and Ornamental Birds (RP1893)

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 FSA in January 2023 from Evonik Operations GmbH ("the applicant") for an authorisation of extension of use of the additive containing *Bacillus velezensis* CECT 5940 (Ecobiol®) under the category 'zootechnical feed additive', functional group 'gut flora stabilizer' for turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening and reared for laying and ornamental birds (except for reproduction) at a minimum recommended dose of 1×10^9 CFU/kg (colony forming units) complete feed.

Under assimilated Regulation (EU) No 2020/1395 (the authorisation of *Bacillus amyloliquefaciens* CECT 5940) the additive is authorised as a zootechnical additive for use in chickens for fattening and chickens reared for laying. This opinion considers the request for the extension of use of the additive in turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening and reared for laying, and ornamental birds (except for reproduction).

The product being assessed is based on viable spores from a strain initially identified and authorised as *Bacillus amyloliquefaciens*, which has since been reclassified as *B. velezensis* CECT 5940. This bacterial strain is deemed suitable for the Qualified Presumption of Safety (QPS) approach. The identity of the active agent, *B. velezensis* CECT 5940, has been confirmed and the compliance with the other qualifications confirmed. Therefore, it was presumed safe for the target species, consumers, and the environment. There are no anticipated concerns regarding the other

components of the additive, leading to the conclusion that Ecobiol® is also considered safe for the target species, the consumers and the environment.

The additive is not an irritant to skin or eyes and is not a skin sensitizer, although it should be considered a respiratory sensitiser. Previous efficacy data enabled the FEEDAP Panel to determine that the additive has the potential to be efficacious at the level of 1×10^9 CFU/kg of feed in the target species.

The FSA/FSS has reviewed the applicant's extension of use application, supporting documentation, and other regulators' risk assessments, most notably the EFSA risk assessment opinion and consider sufficient evidence has been demonstrated to conclude without 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 submitted by Evonik Operations GmbH (Rodenbacher Chaussee 4, 63457 Hanau, Germany) consisting of *Bacillus velezensis* CECT 5940 (Ecobiol®) under Assimilated Regulation (EC) No 1831/2003 (EC, 2003) in each nation of Great Britain (GB) for an authorisation of an extension of use of the additive under the category 'zootechnical feed additive', functional group 'gut flora stabilizer' for turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening and reared for laying and ornamental birds (except for reproduction) at a minimum recommended dose of 1 x 10^9 CFU/kg complete feed. *Bacillus velezensis* CECT 5940 (Ecobiol®, identification number 4b1822), has been authorised in GB as a feed additive for chickens for fattening and chickens reared for laying under Commission Implementing Regulation (EU) No 2020/1395 (EC, 2020), therefore, this opinion only considers the requested new use of the additive.

In line with Article 8 of 1831/2003 (EC, 2003), the assessment has considered whether 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 of the product consisting of *B. velezensis* CECT 5940 (Ecobiol®) for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by the 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, 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 2021, EFSA published a risk assessment opinion on the new proposed conditions of use of *Bacillus velezensis* CECT 5940 (Ecobiol®) (EFSA, 2021). 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 velezensis CECT 5940	Feed additive	Zootechnical feed additive, gut flora stabiliser	a minimum recommended dose of 1×10^9 CFU/kg complete feed.

2. Assessment

2.1. Details of other regulators' opinions

2.1.1. Previous authorisations and opinions

Under Commission Implementing Regulation (EU) No 2020/1395, the additive is authorised as a zootechnical additive for use in chickens for fattening and chickens reared for laying.

In 2021, EFSA published a risk assessment opinion on the new use of the conditions of the authorisation of *Bacillus velezensis* CECT 5940 (Ecobiol®) for its use as a feed additive (EFSA, 2021). The opinion described the additive as: being safe for the target species, consumers and the environment; being a respiratory sensitiser but not an irritant to skin and eyes or a skin sensitiser; having the potential to be efficacious in turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening and reared for laying and ornamental birds (except for reproduction).

2.1.2. Methodology applied in the EFSA opinion

The FEEDAP assessed the safety and the efficacy of the additive consisting of *Bacillus velezensis* CECT 5940 (Ecobiol®), in accordance with guidance documents:

- Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017c);
- Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012);

- Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b);
- 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 additives for the consumer (EFSA FEEDAP Panel, 2017a);
- Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a);
- Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019);

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

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

2.2.1. Characterization of the additive

The additive, Ecobiol®, is currently authorised as a powder in three forms:

- Ecobiol® with a minimum concentration of *Bacillus velezensis* CECT 5940 of 1 x 10^9 CFU/g additive.
- Ecobiol® 500 with a minimum concentration of *Bacillus velezensis* CECT 5940 of 2 x 10^9 CFU/g additive.
- Ecobiol® Plus with a minimum concentration of *Bacillus velezensis* CECT 5940 of 1 x 10^{10} CFU/g additive.

All three forms of the additive consist of the spore concentrate (1-10%) and calcium carbonate (90-99% by weight); all forms have previously been fully characterised by EFSA (EFSA, 2008; EFSA FEEDAP Panel, 2020). No changes have been made to the manufacturing process and compositions of the additive since the previous applications; therefore, the previous data relating to the impurities, physical properties, shelf life, stability and capacity to homogeneously disperse in feed provided still apply to this opinion. New data on the microbial contamination of the additive was provided in three batches for Ecobiol® and Ecobiol® Plus. The results were below the limit of detection (LOD): yeasts (< 100 CFU/g), moulds (< 100 CFU/g), *Escherichia coli* (< 1 CFU/g), *Enterobacteriaceae* (< 10 CFU/g), *Salmonella* spp. (not detected in 25 g), *Clostridium* spp. (most probable number (MPN) < 1.8/g in all batches except for one batch of Ecobiol® Plus where MPN was 2.0/g) and *Bacillus cereus* (< 100 CFU/g).

FSA and FSS agree with the conclusions reached for the characterisation of the additive. The data was 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 are not changed by 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. Characterisation of the active agent

The active agent was deposited in the Spanish Type Culture Collection with the accession number CECT 5940; it was not genetically modified, and no plasmids were found.

A genome-wide average nucleotide identity (gANI) analysis of the whole genome sequence (WGS) against 5,034 *Bacillus* spp. genomes confirmed identity of the strain. The strain was found to be susceptible to relevant antibiotics and was reported to lack acquired antimicrobial resistance genes and cytotoxicity potential (EFSA FEEDAP Panel, 2020). The strain was initially identified and authorised as *Bacillus amyloliquefaciens* and has since been reclassified as *B. velezensis* CECT 5940.

In the current assessment, the applicant used relevant databases to perform a new search, confirming the previous results as any genes of concern were identified.

The production of aminoglycosides by *B. velezensis* CECT 5940 was evaluated with no observed inhibition, indicating lack of aminoglycoside production.

The FSA and FSS agree with the conclusions reached for the characterisation of the active agent. The data was 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 identity and the manufacturing process of the additive are not changed by the current application made to the FSA and FSS and as such has not been subject to further assessment. The characterisation of the active agent is provided as per the existing authorisation and as assessed by EFSA.

2.2.3. Condition of use

The additive has a minimum proposed level of 1 x 10^9 CFU/kg complete feed. It is intended to be used in complete feed for turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening and reared for laying and ornamental birds (except for reproduction).

The applicant requested same conditions of use as EFSA evaluated in their latest opinion (EFSA, 2021). The FSS and FSA agree with the proposed conditions of use.

2.2.4. Conclusion on Section II

The additive was fully characterised, the identity of the active agent and the conditions of use of the additive were established in the previous opinion (EFSA, 2008; EFSA FEEDAP Panel, 2020), and the Panel considered the data from the previous opinion valid for the current assessment.

The FSA and FSS agree with the conclusions reached for the characterisation of the additive and active agent and the conditions of use.

2.3. Section III: Safety

During the current assessment, the active agent was reclassified as *B. velezensis*, which EFSA considers suitable for the QPS approach (EFSA BIOHAZ Panel, 2020; EFSA, 2007). This requires conclusive strain identification, evidence of the absence of acquired antimicrobial resistance genes, as well as confirmation of no toxigenic potential and no capacity to produce aminoglycosides. In the previous EFSA assessment, the lack of toxigenic potential and acquired resistance determinants to antibiotics was confirmed (EFSA FEEDAP Panel, 2020).

For this assessment, the identity of *B. velezensis* CECT 5940 was established and the compliance with the other qualifications of this species were confirmed. Therefore, the active agent is presumed safe for target species, consumers, and the environment. The other component, calcium carbonate, also raises no concerns. The additive is also considered safe for the target species, the consumer and the environment. This safety conclusion extends to new species and categories requested for use (turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening and reared for laying and ornamental birds (except for reproduction)).

The additive was previously determined not to be an irritant to skin and eyes or a dermal sensitiser but should be considered a respiratory sensitiser. Susceptibility to the relevant antibiotics and lack of toxigenic potential were also established. No new data was provided to reconsider

previous conclusions (EFSA, 2008; EFSA FEEDAP Panel, 2020). An updated literature review from 2007 to 2020 found no issues of concern for the safety of the additive.

The FSA and FSS agree with the conclusions reached on the data and the use of qualified presumption of safety (QPS), as this approach has been previously used in GB and it follows the FSA and FSS published guidance. The FSA and FSS agree with the conclusions reached for the safety of the user. 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 FSA and FSS agree with the view that the new use do not introduce new risk to safety for user. The FSA and FSS agree with the extrapolation for turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening and reared for laying and ornamental birds (except for reproduction), which is supported by the guidance that is also applicable in GB, based on physiological similarities between species.

2.3.1. Conclusion on Section III

The additive is considered safe for the target species, the consumer and the environment. The additive was previously determined not to be an irritant to skin and eyes or a dermal sensitiser but should be considered a respiratory sensitiser. As the FEEDAP Panel was not aware of any new information that would lead it to reconsider the previous conclusions, those conclusions remain valid for the current assessment. The requested change in conditions for use does not impact the safety of the additive, according to the FEEDAP Panel.

2.4. Section IV: Efficacy

The efficacy of the additive has been confirmed at 1 x 10^9 CFU/kg of complete feed for chickens for fattening (EFSA, 2008). These findings can be extrapolated to the avian species proposed in the current application when used under the same conditions. Consequently, the Panel concluded that the additive has the potential to be efficacious in turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening and laying, and ornamental birds (excluding reproduction) at a minimum level of 1 x 10^9 CFU/kg of complete feed.

The FSA and FSS agree with these conclusions and with the approach of extrapolating from chickens for fattening to turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening and laying, and ornamental birds (excluding reproduction). This is supported by the guidance that is also applicable in GB, based on principles of similarity between species' metabolism and digestive tracts. This demonstrates that

Bacillus velezensis CECT 5940 favourably affects animal production, performance or welfare, particularly by affecting the gastrointestinal flora of the target species.

3. Analytical method evaluation

FSA/FSS evaluated the EURL analytical method evaluation, noting it was carried out in 2010, 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, 2009). FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The FEEDAP Panel concluded that *Bacillus velezensis* CECT 5940 (Ecobiol®) remains safe for target species, consumers and the environment, under the conditions of authorisation. The Panel concluded that the additive is not irritant to skin and eyes or skin sensitiser but should be considered a respiratory sensitiser. The requested change in conditions for use does not impact the safety of the additive, according to the FEEDAP Panel.

The FEEDAP Panel concluded that the additive has the potential to be efficacious in turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening and laying, and ornamental birds (excluding reproduction) at a minimum level of 1×10^9 CFU/kg of complete feed.

5. Caveats and uncertainties

No cavities and uncertainties were identified.

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 the extension of the use of the feed additive consisting of *Bacillus velezensis* CECT 5940 (Ecobiol®) for turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening and reared for laying, and ornamental birds (excluding reproduction); 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 extension of use. The conclusions of the EFSA opinion have been reviewed in detail by 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
ARG-ANNOT	Antibiotic Resistance Gene-ANNOTation
CARD	Comprehensive Antibiotic Resistance Database
CFU	Colony forming Unit
EC	European Commission
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FSA	Food Standards Agency
FSS	Food Standards Scotland
gANI	Genome-wide average nucleotide identity
GB	Great Britain
LOD	Limit of detection
QPS	Qualified Presumption of Safety
RP	Regulated Product
UK	United Kingdom
WGS	Whole genome sequence

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