REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment on the Safety and Efficacy of an Additive of *Enterococcus faecium* DSM 2121913, *Bifidobacterium animalis spp. animalis* DSM 16284 and *Ligilactobacillus salivarius* DSM 16351 (Biomin® C3) as a Feed Additive for Its Use in All Poultry Species for Fattening and Reared for Laying/ Breeding (RP1603)

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An application was submitted to the Food Standards Agency in June 2022 from BIOMIN GmbH ('the applicant') for the renewal authorisation of an additive consisting of *Enterococcus faecium* DSM 21913, *Bifidobacterium animalis ssp. animalis* DSM 16284 and *Ligilactobacillus salivarius* DSM 16351 (Biomin® C3), under the category of 'zootechnical additive' and functional group 'gut flora stabiliser' for its use in chickens for fattening, chickens reared for laying and minor poultry species other than laying and for the extension of use of the additive under the same category for its use in all poultry species for fattening and reared for laying/breeding. The additive is proposed to be used at a minimum inclusion rate of 1×10^8 CFU/kg in complete feed or 5×10^7 CFU/L in drinking water.

The FEEDAP Panel concluded that Biomin® C3 remains safe for chickens for fattening, chickens reared for laying, and minor poultry species other than laying, as well as for consumers and the environment, under the authorized conditions of use.

The Panel concluded that extending the use of the additive to all poultry for fattening and reared for laying/breeding is safe for the target species, consumers, and the environment. The additive is efficacious at a minimum inclusion level of 1×10^8 CFU/kg in complete feed and 5×10^7 CFU/L in water.

The additive should be considered as a potential respiratory sensitiser but as non-irritant to eyes and skin. Conclusions could not be drawn on the potential to cause skin sensitisation.

FSA/FSS has reviewed the applicant's renewal and 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 document represents the opinion of the FSA and FSS.

1. Introduction

In accordance with Retained EU Regulation 1831/2003 (EC, 2003) on feed additives, the applicant, (BIOMIN GmbH, Erber Campus 1, 3131 Getzersdorf, Austria), submitted an application *of Enterococcus faecium* DSM 21913, *Bifidobacterium animalis ssp. animalis* DSM 16284 and *Ligilactobacillus salivarius* DSM 16351 (Biomin® C3) under the category of 'zootechnical additive' and functional group 'gut flora stabiliser' for: renewal of authorisation for its use in chickens for fattening, chickens reared for laying and minor poultry species other than laying; extension of use in all poultry species for fattening and reared for laying/breeding.

Whilst it was a Member State of the EU, the UK accepted the risk assessments of the European Food Safety Authority (EFSA) in respect of authorisations for regulated food and feed products. When GB left the EU, it retained the same regulations for food and feed regulated products; FSA and FSS also adopted equivalent technical guidance and quality assurance processes to be able to undertake GB risk assessments for regulated product applications.

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 opinion, to provide a summary assessment of the evidence of safety presented in this report.

In 2023, EFSA published an opinion (EFSA FEEDAP Panel, 2023) on the renewal of application of Biomin® C3, for its use as a feed additive, including the proposed extensions of use. The Commission subsequently re-authorised the product with the extensions for use. 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.

Specifically, in reviewing the assessment that EFSA have recently completed, the reviewers have 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. Consideration has been given to the processes undertaken

to ensure the EFSA opinion is robust and whether there are any aspects that would require further review, such as specific issues for the countries of GB. The result of the assessment is that there is sufficient evidence of safety to conclude without requiring further assessment at this time.

The result of the assessment is that there is sufficient evidence of safety for the UK to conclude this assessment at this time. This document 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
Biomin® C3	Feed additive	Zootechnical additive	All poultry species for fattening and reared for laying/ breeding

2. Details of other Regulators Opinions

The additive Biomin® C3, has previously been authorised in the EU by Commission implementing regulation (EU) 2024/1200 (EC, 2024). In 2023, EFSA published an opinion (EC, 2003) on the renewal of application of Biomin® C3, for its use as a feed additive. This opinion has been reviewed by FSA/FSS risk assessors.

2.1. Methodology applied in the EFSA Opinion

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) assessed the safety and the efficacy of Biomin® C3, in accordance with guidance documents: Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b), Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a) and principles in Regulation (EC) No 429/2008 (EC, 2008).

2.1.1. Characterization of the additive

The current authorization for the additive requires a minimum content of the active agents in colony forming units (CFU)/g of the additive of 3×10^9 *B. animalis* DSM 16284, 1×10^9 *L. salivarius* DSM 16351 and 6×10^9 *E. faecium* DSM 21913 (ratio 3:1:6). The total bacterial cell count was 1.5×10^{10} (range 1.4-1.7 $\times 10^{10}$) CFU/g with an average of 3.9×10^9 CFU/g additive (range 3.2-4.2 $\times 10^9$) for *B. animalis* DSM 16284, 1.5×10^9 (range

1.3-1.9 × 10^9) CFU/g additive for *L. salivarius* DSM 16351 and 9.6 × 10^9 CFU/g additive (range 8.7-11.5× 10^9) for *E. faecium* DSM 21913 as shown in analysis of 5 batches of the additive.

It was stated by the applicant that the manufacturing process was not modified since the initial authorization in 2013. The product is composed of 48% bacterial cells with the following composition: 3% w/w *B. animalis* DSM 16284, 5% w/w *L. salivarius* DSM 16351, 40% w/w *E. faecium* DSM 21913, and up to 52% of carrier (sucrose, maize starch and water). The product also contains the following excipients: cryoprotectants (1.5% w/w inulin) and coating substances (4% w/w hydrogenated vegetable oil, 0.2% w/w xanthan gum, 0.02% w/w hydroxypropyl methyl cellulose and 0.02% w/w methyl cellulose).

Analysis of 3 batches of the additive demonstrated compliance with predefined specifications: *Escherichia coli* (<10 CFU/g), *Salmonella spp.* (not detected in 25 g), yeasts and filamentous fungi (<1000 CFU/g), coliforms (<1000 CFU/g) and *Enterobacteriaceae* (<1000 CFU/g). The mycotoxin analysis of 3 batches, including aflatoxins (B1, G1, B2, G2), deoxynivalenol, zearalenone, ochratoxin A, fumonisin B1 and B2, HT-2 toxin, and T-2 toxin, showed that all levels were below the detection limits (LOD) of the analytical methods. In addition, testing of 3 batches for arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) showed values below the limit of quantification (LOQ) of the analytical methods.

Analysis of 3 batches showed mean dusting potential of 362 mg/m^3 (range 300-420 mg/m^3) using Stauber–Heubach method.

As the formulation and manufacturing process of the additive have remained the same, the homogeneity and stability data from the previous evaluation are considered valid for the current assessment (EFSA FEEDAP Panel, 2012).

2.1.2. Characterization of the active agents

2.1.2.1. Enterococcus faecium DSM 21913

The strain DSM 21913, originally isolated from the digestive tract of a healthy farm animal and was not genetically modified. The strain was taxonomically identified as *E. faecium* by analysing whole genome sequence (WGS) using Average Nucleotide Identity (ANI) analysis. The OrthoANI value of 99% was compared to the type strain *E. faecium* DSM 20477^T. Further confirmation was made through alignment-free genome distance estimation and phylogenetic analysis of 35 core genes, revealing that *E. faecium* strains were the were the closest genomes.

The strain's WGS was examined for the presence of plasmids by cross-referencing against a relevant database, identifying one putative sequence. The strain's WGS was examined for the presence of antimicrobial resistance (AMR) genes by cross-referencing against two databases. No concerns were identified from the search at nucleotide and/or protein level with set thresholds of 80% identity and 70% length coverage.

The presence of genes encoding for virulence factors was examined using the WGS of the strain against a relevant database. No hits of concern were identified and presence of IS*16*, *hyl*Efm and *esp* genes in the strain was ruled out.

The microdilution assay was used to assess the strain's susceptibility to antimicrobials. The strain is considered susceptible to all relevant antibiotics, including ampicillin, because all the minimum inhibitory concentration (MIC) values were equal or below the specified cut-off values stated in the EFSA FEEDAP Guidance (EFSA FEEDAP Panel, 2018b).

A disc-diffusion agar method against the reference strains: *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 confirmed no antimicrobial activity.

2.1.2.2. Bifidobacterium animalis DSM 16284

The strain DSM 16284, originally isolated from the digestive tract of a healthy farm animal, has not been genetically modified and was taxonomically identified as *B. animalis ssp. Animalis.* The identification was based on an OrthoANI value of 98.7% with the type strain *B. animalis ssp. animalis ATCC 25527*^{T}. Further confirmation was made through alignment-free genome distance estimation and phylogenetic analysis of 117 core genes, revealing that *B. animalis ssp. animalis* strains were the closest genomes.

No hits of concern were identified during examination of the WGS data of the strain for the presence of AMR genes.

The microdilution assay was used to assess the strain's susceptibility to antimicrobials. The strain is considered susceptible to all relevant antibiotics because all the MIC values were equal or below the specified cut-off values stated in the EFSA FEEDAP Guidance (EFSA FEEDAP Panel, 2018b). Clindamycin exceeded the cut-off value by one dilution, which was considered within the normal range variation of the method by the FEEDAP Panel.

2.1.2.3. Ligilactobacillus salivarius DSM 16351

The strain DSM 16351, originally isolated from the digestive tract of a healthy farm animal and has not been genetically modified. The strain was taxonomically identified as *L. salivarius* by analysing whole genome sequence (WGS) using ANI analysis. The OrthoANI value of 97.2% was compared to the type strain *L. salivarius* DSM 20555^T. Further confirmation was made through alignment-free genome distance estimation and phylogenetic analysis of 41 core genes, revealing that *L. salivarius* strains were the were the closest genomes.

The strain's WGS was examined for the presence of plasmids by cross-referencing against a relevant database, identifying 6 complete or partial plasmid sequences.

No concerning hits were identified during examination of the WGS data of the strain, including plasmids, for the presence of AMR genes.

The microdilution assay was used to assess the strain's susceptibility to antimicrobials. The strain is considered susceptible to all relevant antibiotics, because all the MICs values were equal or below the specified cut-off values stated in the EFSA FEEDAP Guidance (EFSA FEEDAP Panel, 2018b).

2.1.3. Conditions of use

A minimum content of 1×10^8 CFU/kg in complete feed or 5×10^7 CFU/L in drinking water is currently authorized in the additive for chickens for fattening, chickens reared for laying, and minor poultry species other than laying.

The following specification are listed under the provisions of the authorisation:

- Storage temperature, storage life and stability to pelleting should be indicated in the directions for use of the additive and premixture.
- The use is permitted in feed containing the following coccidiostats: maduramicin ammonium, diclazuril, robenidine hydrochloride, decoquinate, narasin, nicarbazin or narasin/nicarbazin.
- Feed containing the permitted coccidiostats can be used simultaneously with the water for drinking containing the additive. However, simultaneous use with antibiotics shall be avoided.
- The additive should be homogenously dispersed in the drinking water.

- During the handling, personal protective equipment such as breathing protection, eye protection and gloves should be used.

No changes to these conditions of use were proposed and the applicant wishes to extend these conditions of authorisation to include all poultry for fattening or reared for laying/breeding.

2.1.4. Safety

No adverse effects have been reported since the approval of the additive according to the applicant.

It was concluded that following the Qualified Presumption of Safety (QPS) approach to safety assessment, the *B. animalis DSM 16284 and L. salivarius DSM 16351* were presumed safe for target species, consumers, and the environment. Based on the provided evidence, the antibiotic resistance qualifications have been met and the identity of the strains have been confirmed. The *E. faecium* strain was found not to belong to the hospital-associated clade, nor did it exhibit resistance to clinically relevant antibiotics. Its metabolic end products are typical of lactic acid bacteria and do not pose any concerns. Consequently, no concerns for the target species, consumers, or the environment are expected from the use of *E. faecium* DSM 21913 in animal nutrition.

No new data have been provided to reconsider previous conclusions on safety of the user. Based on a Buhler test following OECD guideline 406, the Panel previously concluded that the additive is not a skin sensitizer. However, it was noted that current OECD test guidelines are designed for chemical substances only, and there are no validated assays available for assessing the sensitization potential of microorganisms. In conclusion, Biomin® C3 can be considered non-irritant to eyes or skin but should be treated as a potential respiratory sensitiser. No conclusions can be made regarding the skin sensitization potential of the additive. It is recommended to use gloves, glasses and breathing protection during handling.

A first literature search covering the period 2015-2021 was performed to provide evidence of safety of the additive under the approved conditions involving use of 7 different databases, with no publications considered relevant for the assessment. Due to limitations in the initial literature review, which did not cover the period since the original authorization of the additive and lacked a list of publications, a second literature search was carried out. This following search used 6 databases covering the period from 2013 to March 2023. None of the 6 hits were considered relevant for the assessment.

2.1.4.1. Safety regarding extension of the use

The extension of use of the additive to all poultry for fattening or reared for laying/breeding was requested in the current application. The Panel concluded that the additive was safe for the target species under the proposed conditions of use based on the presumed safety of the *B. animalis and L. salivarius* strains, the absence of pathogenicity of *E. faecium* DSM 21913 and the results of two tolerance studies provided in chickens for fattening in the previous opinions. One tolerance study supported the safety of the additive in feed (EC, 2024), while the second study supported extension of the authorisation to feed and drinking water for chickens reared for laying and minor poultry species up to the point of lay (EFSA FEEDAP Panel, 2015).

The conclusions on the safety of the additive in chickens for fattening can be extended to all poultry species for fattening or reared for laying/ breeding at the same conditions of use and the Panel concluded that the extension of use would not introduce risks for consumers, users and the environment as evaluated in the previous opinions.

2.1.5. Efficacy

An assessment of the efficacy for the renewal of the authorisation was not needed as there was no proposal to amend or supplement the conditions of use for already authorised species. The FEEDAP Panel concluded that the outcomes previously reached for chickens for fattening can be extrapolated to all poultry for fattening or reared for laying/breeding at the same inclusion levels, including the use of authorized coccidiostat.

3. Analytical method evaluation

FSA/FSS accepts the EURL analytical method evaluation report (EURL-FA, 2010). FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Other Regulators Opinions and Conclusions

The FEEDAP Panel concluded that Biomin® C3 remains safe for chickens for fattening, chickens reared for laying, and minor poultry species other than laying, as well as for consumers and the environment, under the authorized conditions of use.

The FEEDAP Panel concluded that extending the use of the additive to all poultry for fattening and reared for laying/breeding is safe for the target species, consumers, and the environment. The additive is efficacious at a minimum inclusion level of 1×10^8 CFU/kg in complete feed and 5×10^7 CFU/L in water.

The additive should be considered as a potential respiratory sensitiser but as non-irritant to eyes and skin. Conclusions could not be drawn on the potential to cause skin sensitisation.

5. Caveats and uncertainties

The current authorization allows the use of the additive without any specified maximum content.

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

6. FSA/FSS conclusions for GB safety assessment

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 submitted to the FSA and FSS, whilst this is only briefly summarised this description is consistent with the conclusions. The conclusions of the EFSA opinion have also been reviewed in detail by FSA and FSS and are considered appropriate and consistent within the identified caveats and uncertainties identified in the opinion and would be applicable to GB. Sufficient evidence has been demonstrated to conclude without further questions or assessment.

7. Outcome of assessment

FSA/FSS has reviewed the applicant's extension of use application, supporting documentation, and other regulators assessments, most notably the EFSA opinion (EC, 2003) and consider sufficient evidence has been demonstrated to conclude without further questions or assessment.

The FSA and FSS conclude that the Biomin® C3 feed additive, as described in this application, is safe and is not liable to have an adverse effect on the target species, worker safety, environmental safety and human health at the intended concentrations of use.

In making this assessment, the following principles have been applied:

1) There is not a legal duty to perform a separate risk assessment for GB and therefore, there was sufficient scientific evidence to make a conclusion on safety with no further questions to the applicant, and therefore no further risk assessment activities are necessary.

2) Sufficient evidence was available in the literature, for example, where other National food safety authorities had positively assessed the application using the same risk assessment guidance in principle and legal requirements in GB with the exception to changes in the General Food Law.

3) Applicants provided sufficient relevant information as requested by FSA/FSS.

4) The FSA and FSS review did not find any issues of divergence from guidance or mutual approaches or new scientific issues for consideration.

5) There were no other specific issues that would require an assessment for the UK or the nations of the UK.

Abbreviations

Acronym	Definition
AMR	Antimicrobial resistance
ANI	Average Nucleotide Identity
CAS	Chemical Abstracts Service
CFU	Colony forming units
EC	European Commission
EU	European Union
EFSA	European Food Safety Authority
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FSA	Food Standards Agency
FSS	Food Standards Scotland
GB	Great Britain
LOQ	Limits of quantification
MIC	Minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
QPS	Quality Presumption of Safety
RP	Regulated Product
UK	United Kingdom
WGS	Whole genome sequence

References

EC (European Commission). (2003). *Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition*. Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on Additives for Use in Animal Nutrition (Text with EEA Relevance) (Legislation.Gov.Uk).

EC (European Commission). (2008). *Regulation No 429/2008 of the European Parliament and of the Council on additives for use in animal nutrition*. 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 (Text with EEA Relevance) (Legislation.Gov.Uk).

EC (European Commission). (2024). *Regulation No 2024/1200 of the European Parliament and of the Council on additives for use in animal nutrition*. Implementing Regulation - EU - 2024/1200 - EN - EUR-Lex (Europa.Eu).

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2012). Scientific Opinion on the safety and efficacy of Biomin C3 (Enterococcus faecium, Bifidobacterium animalis and Lactobacillus salivarius) for chickens for fattening. *EFSA Journal*, *10*(12), 2965. https://doi.org/10.2903/j.efsa.2012.2965

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2015). Scientific Opinion on the safety and efficacy of Biomin®C3 (Bifidobacteriumanimalis ssp. Animalis, Lactobacillussalivarius ssp. Salivarius and Enterococcusfaecium) as feed additive for chickens for fattening, chickens reared for laying and minor avian species other than laying species. *EFSA Journal*, *13*(1), 3966. https://doi.org/10.2903/j.efsa.2015.3966

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2017a). Guidance on the assessment of the safety of feed additives for the target species. *EFSA Journal*, *15*(10), 5021.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2017b). Guidance on the identity, characterisation and conditions of use of feed additives. *EFSA Journal*, *15*(10), 5023.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2018a). Guidance on the assessment of the efficacy of feed additives. *EFSA Journal*, *16*(5), 5274.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2018b). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*, *16*(3), 5206.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2021). Guidance on the renewal of the authorisation of feed additives. *EFSA Journal*, *19*(1), 6340.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2023). Scientific Opinion on the Assessment of the feed additive consisting of Enterococcus faecium DSM 21913, Bifidobacterium animalis DSM 16284 and Ligilactobacillus salivarius DSM 16351 (Biomin® C3) for chickens for fattening, chickens reared for laying and minor poultry species other than laying for the renewal of its authorisation and extension of use in all poultry species for fattening and reared for laying/breeding (Biomin GmbH). *EFSA Journal*, *21*(12), 8356. https://doi.org/10.2903/j.efsa.2023.8356

EURL-FA (European Reference Laboratory for Feed Additives). (2010). *CRL Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of Feed Additive according to Regulation (EC) No 1831/2003*. FinRep-FAD-2009-0034 (Europa.Eu).