SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of *Enterococcus faecium* DSM 33761, *Pediococcus acidilactici* DSM 33758, *Bifidobacterium animalis* DSM 16284, *Limosilactobacillus reuteri* DSM 33751 and *Ligilactobacillus salivarius* DSM 16351 (Biomin® C5) for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species for fattening and reared for laying/breeding (Biomin GmbH)

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Abstract

Following a request from the European Commission, the EFSA 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 a feed additive consisting of viable cells of *Enterococcus faecium* DSM 33761, *Pediococcus acidilactici* DSM 33758, *Bifidobacterium animalis* DSM 16284, *Limosilactobacillus reuteri* DSM 33751 and *Ligilactobacillus salivarius* DSM 16351 (Biomin® C5) as a zootechnical additive (functional group: gut flora stabiliser) for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species for fattening and reared for laying/breeding. Biomin® C5 is marketed in two formulations: a coated and a non-coated formulation with a total minimum microbial count of 1×10^{11} and 4×10^{10} colony forming unit (CFU)/g product, respectively. The Panel considered that the use of Biomin® C5 in feed at the proposed conditions of use raises no risk for the target species, consumers and the environment. Both coated and non-coated formulations of Biomin® C5 are considered respiratory and skin sensitisers, but not skin irritants. The FEEDAP Panel was not in the position to conclude on the eye irritation potential of any formulation. Due to lack of sufficient data, the FEEDAP Panel could not conclude on the efficacy of the additive in the target species at the proposed conditions of use. Biomin® C5 is compatible with nicarbazin, diclazuril, decoquinate and halofuginone. No conclusions can be drawn on the compatibility of Biomin® C5 with monensin sodium, robenidine hydrochloride, maduramicin ammonium and lasalocid A sodium.

Keywords: zootechnical additive, *Enterococcus faecium* DSM 33761, *Pediococcus acidilactici* DSM 33758, *Bifidobacterium animalis* DSM 16284, *Limosilactobacillus reuteri* DSM 33751, *Ligilactobacillus salivarius* DSM 16351, Biomin® C5

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] 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 feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Biomin GmbH[2] for the authorisation of the product containing *Enterococcus faecium* DSM 33761, *Pediococcus acidilactici* DSM 33758, *Bifidobacterium animalis* DSM 16284, *Limosilactobacillus reuteri* DSM 33751 and *Ligilactobacillus salivarius* DSM 16351 (Biomin® C5), when used as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species other than laying species (category: zootechnical; 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 4(1) (authorisation of a feed additive or new use 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 2 September 2022.

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, consumers, user and the environment and on the efficacy of the feed additive consisting of *E. faecium* DSM 33761, *P. acidilactici* DSM 33758, *B. animalis* DSM 16284, *L. reuteri* DSM 33751 and *L. salivarius* DSM 16351 (Biomin® C5), when used under the proposed conditions of use (see **Section 3.1.5**).

1.2. Additional information

The product under assessment is based on viable cells of *E. faecium* DSM 33761, *P. acidilactici* DSM 33758, *B. animalis* DSM 16284, *L. reuteri* DSM 33751 and *L. salivarius* DSM 16351 and is not authorised as a feed additive in the European Union.

EFSA has published two opinions on another feed additive, currently authorised (Biomin® C3), containing two of the strains included in the product under assessment, *B. animalis* DSM 16284 and *L. salivarius* DSM 16351 (EFSA FEEDAP Panel, 2012a, 2015).

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[3] in support of the authorisation request for the use of *Enterococcus faecium* DSM 33761, *Pediococcus acidilactici* DSM 33758, *Bifidobacterium animalis* DSM 16284, *Limosilactobacillus reuteri* DSM 33751 and *Ligilactobacillus salivarius* DSM 16351 (Biomin® C5), as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from the 2 September 2022 to 5 December 2022 for which the received comments were considered for the assessment.

In accordance with Article 38 of the Regulation (EC) No 178/2002[4] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Erber Campus 1, 3131 Getzersdorf, Austria.
[3] Dossier reference: FEED-2022-3870.
[4] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

concerning transparency and confidentiality,[5] a non-confidential version of the dossier has been published on Open.EFSA.[6]

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations,[5] EFSA carried out a public consultation on the non-confidential version of the application from 28 February to 21 March 2023 for which no comments were received.

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, peer-reviewed scientific papers, other scientific reports and experts' (elicitation) knowledge, to deliver the present output.

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.[7]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Biomin® C5 is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), 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 environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The product under assessment (herein and after referred to as Biomin® C5) consists of a preparation of viable cells of *E. faecium* DSM 33761, *P. acidilactici* DSM 33758, *B. animalis* DSM 16284, *L. reuteri* DSM 33751 and *L. salivarius* DSM 16351, and is intended to be used as a zootechnical additive (functional group: gut flora stabilisers) in feed and water for drinking for all growing poultry, including chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species other than laying species.

3.1. Characterisation

3.1.1. Characterisation of the active agents

3.1.1.1. *Enterococcus faecium* DSM 33761

The *E. faecium* strain was originally isolated from the gut of a healthy chicken and is deposited in Deutsche Stammsammlung von Mikroorganismen und Zellkulturen (DSMZ) under the accession number DSM 33761.[9] It has not been genetically modified.

The taxonomical identification of the active agent as *E. faecium* was confirmed by a bioinformatic analysis using the whole genome sequence (WGS) data. The taxonomic assignment was based on an average nucleotide identity (ANI) of 99.7% when compared to the type strain *E. faecium* NBRC 100486^T (whereas similarity with *E. lactis* LMG 25958^T was 94.6%) as well as by alignment-free genome distance estimation and phylogenetic analysis using 32 core genes which showed that *E. faecium* strains were the closest genomes.[10]

The susceptibility of the active agent to antimicrobials was tested using a microdilution method and including the battery of antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2018b).[11] All the

[5] Decision available online: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements
[6] Available online: https://open.efsa.europa.eu/dossier/FEED-2022-3870
[7] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/feed-2022-3870_en
[8] 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.
[9] Annex II_02.
[10] Annex II_43_amended.
[11] Annex II_51.

minimum inhibitory concentration (MIC) values were equal to or below the cut-off values for the species. Therefore, the strain is considered to be susceptible to all the relevant antibiotics.

The WGS data of the active agent was interrogated for the presence of antimicrobial resistance (AMR) genes by a search against the [redacted] (thresholds: 80% identity, 70% length of the subject sequence at nucleotide and/or protein level).[10] Hits obtained were the *eat(A)* (encoding an efflux pump (ABC-type transporter), *msrC* (encoding an efflux pump transporter) and *aac(6')* (encoding an aminoglycoside 6'-N-acetyltransferase). All these genes are considered intrinsic in *E. faecium* ([redacted]), and thus, of no concern.

According to the FEEDAP guidance (EFSA FEEDAP Panel, 2018b), the safety of *E. faecium* should be assessed by demonstrating the absence of genetic markers typical of the clinical isolates *E. faecium* clade A (IS*16*, *esp*, *hyl*Efm) and the susceptibility to ampicillin (MIC ≤ 2 mg/L). *E. faecium* DSM 33761 was susceptible to ampicillin (MIC 0.5 mg/L). The WGS data of DSM 33761 were interrogated for the presence of genes encoding for virulence factors using the virulence factor database ([redacted]), and no hits of concern were detected. Moreover, the presence of IS*16*, *hyl*Efm and *esp* genes in the genome of DSM 33761 was excluded [redacted].[12]

Although the species is not expected to produce antimicrobials, the capacity of the active agent to produce antimicrobials was tested in the culture supernatant of *E. faecium* DSM 33761.[13] This was done using a disc-diffusion agar method against the following reference strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212 and *B. subtilis* ATCC 6633. No antimicrobial activity was detected.

3.1.1.2. *Pediococcus acidilactici* DSM 33758

The *P. acidilactici* strain was isolated from the gut of a healthy chicken and is deposited in the DSMZ under the accession number DSM 33758.[14] It has not been genetically modified.

The taxonomical identification of the active agent as *P. acidilactici* was confirmed by a bioinformatic analysis using the WGS data. The taxonomic assignment was based on ANI of 98.8% when compared to the type strain *P. acidilactici* DSM 20284^T as well as by alignment-free genome distance estimation and phylogenetic analysis using 79 core genes which showed that *P. acidilactici* strains were the closest genomes.[15]

The antimicrobial susceptibility of the active agent to antimicrobials was tested using a microdilution method and including the data set of antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2018b).[16] All the MIC values were equal to or below the cut-off values for the genus. Therefore, the DSM 33758 strain is considered to be susceptible to all the relevant antibiotics.

The WGS data of the active agent *P. acidilactici* DSM 33758 were interrogated for the presence of AMR genes as described for *E. faecium*.[15] No hits of concern were identified.

3.1.1.3. *Bifidobacterium animalis* DSM 16284

The *B. animalis* strain was isolated from the gut of a healthy chicken and is deposited in the DSMZ under the accession strain number DSM 16284.[17] It has not been genetically modified.

The active agent DSM 16284 was taxonomically identified as *B. animalis* ssp. *animalis* based on an OrthoANI value of 98.7% with the type strain *B. animalis* ssp. *animalis* ATCC 25527^T (whereas similarity with *B. animalis* ssp. *lactis* strains was ≤ 95.6%).[18] This was further confirmed by alignment-free genome distance estimation and phylogenetic analysis using 117 core genes which showed that *B. animalis* ssp. *animalis* strains were the closest genomes.

The antimicrobial susceptibility of the active agent DSM 16284 was tested against the battery of antibiotics recommended by the FEEDAP Panel (EFSA FEEDAP Panel, 2018b) by microdilution assay.[19] All the MIC values for the strain were equal to or fell below the corresponding cut-off values, except for clindamycin which was higher than the cut-off value (2 vs. 1 mg/L). A MIC value of one dilution

[12] Annex II_43_amended.
[13] Annex II_50.
[14] Annex II_03.
[15] Annex II_44.
[16] Annex II_52.
[17] Annex II_04.
[18] Annex II_45.
[19] Annex II_53.

higher than the cut-off is within the normal range variation of the method. Therefore, *B. animalis* DSM 16284 is considered susceptible to all relevant antibiotics.

The WGS data of the active agent DSM 16284 was interrogated for the presence of AMR genes as described above for *E. faecium* DSM 33761.[18] No hits of concern were identified.

3.1.1.4. *Limosilactobacillus reuteri* DSM 33751

The *L. reuteri* strain was isolated from the gut of a healthy chicken and is deposited in the DSMZ under the accession number DSM 33751.[20] It has not been genetically modified.

The taxonomical identification of the active agent as *L. reuteri* was confirmed by a bioinformatic analysis using the WGS data. The taxonomic assignment was based on ANI of 96.1% when compared to the type strain *L. reuteri* DSM 20016^T, as well as by alignment-free genome distance estimation and phylogenetic analysis using 53 core genes which showed that *L. reuteri* strains were the closest genomes.[21]

The antimicrobial susceptibility of the active agent to antimicrobials was tested using a microdilution method and including the data set of antimicrobials recommended by EFSA (EFSA FEEDAP Panel, 2018b).[22] All the MIC values were equal to or below the cut-off values for the species. Therefore, the DSM 33751 strain is considered to be susceptible to all the relevant antibiotics.

The WGS data of the active agent *L. reuteri* DSM 33751 were interrogated for the presence of AMR genes as described above for *E. faecium* DSM 33761.[21] No hits were identified.

3.1.1.5. *Ligilactobacillus salivarius* DSM 16351

The *L. salivarius* strain was isolated from the gut of a healthy chicken and is deposited in the DSMZ under the accession number DSM 16351.[23] It has not been genetically modified.

The taxonomic identification of the active agent as *L. salivarius* was confirmed by analysis of its WGS data.[24] This was based on an ANI analysis which showed an OrthoANI value of 97.2% compared to the type strain *L. salivarius* DSM 20555^T, as well as by alignment-free genome distance estimation and phylogenetic analysis using 41 core genes which showed that *L. salivarius* strains were the closest genomes.

The WGS data were screened for the presence of plasmids against the PlasmidFinder database and six contigs showing complete or partial plasmid sequences were identified.[24]

The antimicrobial susceptibility profile of the active agent was tested against the battery of antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2018b) by microdilution assay.[25] All the MIC values for the strain were equal to or fell below the corresponding cut-off values. Therefore, *L. salivarius* DSM 16351 is considered to be susceptible to all relevant antibiotics.

The WGS data of the active agent DSM 16351, including plasmids, were interrogated for the presence of AMR genes as described for *E. faecium* DSM 33761.[24] No hits were identified.

3.1.2. Manufacturing process

[redacted] Thereafter, two different formulations are manufactured:

i) non-coated: the resulting individual strain lyophilisates (26% in total in the final product, representing *E. faecium* and *P. acidilactici* 5.5% (w/w) each and 5% each of the other strains) are mixed with inulin (74%) to reach a final minimum total microbial count of 1.0×10^{11} colony forming unit (CFU)/g product.

ii) coated: the strain lyophilisates are individually coated with hydrogenated vegetable oil, sucrose, hydroxypropyl methyl cellulose and methyl cellulose[26] and subsequently mixed (21% in total, representing *P. acidilactici* and *B. animalis* 4.5% (w/w) each and 4% each of the other strains) with inulin (79%) to reach a final minimum total microbial count of 4.0×10^{10} CFU/g product.

[20] Annex II_05.
[21] Annex II_46.
[22] Annex II_54.
[23] Annex II_06.
[24] Annex II_47.
[25] Annex II_55.
[26] Currently under re-evaluation according to art. 10 of Regulation EC No 1831/2003 by the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP).

3.1.3. Characterisation of the additive

The specifications of the additive proposed by the applicant and expressed as minimum CFU/g additive, are:

- For the non-coated formulation: 3.3×10^{10} *E. faecium* DSM 33761; 3.3×10^{10} *P. acidilactici* DSM 33758; 3.0×10^{10} *B. animalis* DSM 16284, 2.5×10^{9} *L. reuteri* DSM 33751 and 2.5×10^{9} *L. salivarius* DSM 16351.
- For the coated formulation: 1.3×10^{10} *E. faecium* DSM 33761; 1.3×10^{10} *P. acidilactici* DSM 33758; 1.2×10^{10} *B. animalis* DSM 16284; 1.0×10^{9} *L. reuteri* DSM 33751 and 1.0×10^{9} *L. salivarius* DSM 16351.

Analytical data to confirm the specifications regarding the batch-to-batch variation were provided for five batches of each formulation of the additive. Counts for each individual strain were determined using a methodology based on colony morphological differences. To support the capability of the method to discriminate between the two lactobacilli species present in the additive, the applicant provided a 16S rRNA sequencing analysis in which colonies, preliminarily identified based on their morphology, were confirmed as representing either *L. reuteri* or *L. salivarius*.[27]

The mean total microbial cell count of the non-coated formulation was 1.5×10^{11} (range 1.4–1.6×10^{11}) CFU/g.[28] The average content of each strain in the non-coated formulation was 4.9×10^{10} CFU/g (range 4.1–5.3×10^{10}) for *E. faecium* DSM 33761; 4.9×10^{10} CFU/g (range 4.2–5.4×10^{10}) for *P. acidilactici* DSM 33758; 4.3×10^{10} (range 3.6–5.1×10^{10}) CFU/g for *B. animalis* DSM 16284; 3.7×10^{9} (range 3.0–4.5×10^{9}) CFU/g for *L. reuteri* DSM 33751 and 3.7×10^{9} CFU/g (range 3.0–4.2×10^{9}) for *L. salivarius* DSM 16351.

The mean total microbial cell count of the coated formulation was 5.7×10^{10} CFU/g.[29] The average content of each individual strain was 1.8×10^{10} CFU/g (range 1.5–2.2×10^{10}) for *E. faecium* DSM 33761; 1.8×10^{10} CFU/g (range 1.5–2.2×10^{10}) for *P. acidilactici* DSM 33758; 1.7×10^{10} CFU/g (range 1.4–2.0×10^{10}) for *B. animalis* DSM 16284; 1.7×10^{9} CFU/g (range 1.3–2.0×10^{10}) for *L. reuteri DSM* 33751 and 1.8×10^{9} CFU/g (range 1.5–2.3×10^{9}) for *L. salivarius* DSM 16351, which showed compliance with the proposed specifications of the additive.

Three batches of each formulation were analysed for chemical and microbiological contamination. All batches analysed showed values of arsenic, cadmium, lead and mercury below their corresponding limit of quantification (LOQ) of the analytical methods.[30]

The same batches were analysed for the content of aflatoxins B1, B2, G1 and G2, deoxynivalenol, ochratoxin A, zearalenone, Fumonisin B1 and B2, HT-2 toxin and T-2 toxin, showing contents below their corresponding limit of detection (LOD) of the analytical methods.[31]

Microbiological contamination was analysed by the determination of yeasts and filamentous fungi (< 1,000 CFU/g), coliforms (< 1,000 CFU/g), *Enterobacteriaceae* (< 1,000 CFU/g), *Escherichia coli* (< 10 CFU/g) and *Salmonella* spp. (no detection in 25 g).[32]

The bulk density in three batches of each formulation, ranged between 537 and583 kg/m^3 for the non-coated formulation and 542–575 kg/m^3 for the coated formulation.[33]

The dusting potential of three batches of each formulation of the additive was determined using the Stauber–Heubach method and showed average values of 2,278 mg/m^3 (range 1,715–2,795 mg/m^3) in the non-coated formulation and 1,790 mg/m^3 (range 1,330–2,250 mg/m^3) in the coated one.[34]

3.1.4. Stability and homogeneity

The shelf life of the additive was investigated in three batches of each formulation (two replicates each) when stored at 4°C or 22°C in light-protected airtight bags for 12 months. The results for the non-coated formulation showed viability losses of ≤ 0.5 log for all strains except for *L. salivarius*, for

[27] Annex II_SIN_Q2.
[28] Annex II_07 to Annex II_11.
[29] Annex II_012 to Annex II_016.
[30] Annex II_17 to Annex II_22; LOQ (mg/kg): Arsenic < 0.5, cadmium < 0.5, lead < 0.5 and mercury < 0.02.
[31] Annex II_23 and Annex II_24; LOD (μg/kg): Deoxynivalenol 20 μg/kg, Zearalenone 5 μg/kg, Aflatoxin B1 0.5 μg/kg, Aflatoxins B2, G1 and G2 1 μg/kg, Ochratoxin A 0.5 μg/kg, Fumonisin B1 and B2 10 μg/kg, HT-2 Toxin 15 μg/kg and T-2 Toxin 10 μg/kg.
[32] Annex II_25 to Annex II_30.
[33] Annex II_32 to Annex II_37.
[34] Annex II_31.

which losses of 0.52 and 0.65 log were observed in two batches of the non-coated form stored at 22°C. The coated formulation showed no viability losses when stored at 4°C, whereas at 22°C the viability losses were of 0.5 log for *L. reuteri* and *L. salivarius* strains.[35]

The stability of Biomin® C5 was investigated in one batch of the coated form of the additive when added to two chicken premixtures at 1 g/kg and stored at 22°C ± 2 in sealed light-protected and airtight bags for 6 months. No viability losses (< 0.5 log) were observed for *E. faecium*, *P. acidilactici*, *B. animalis*, whilst the losses of *L. reuteri* ranged 0.8–0.9 log CFU/g and of *L. salivarius* 0.6–0.7 log CFU/g.[36]

The stability of the individual Biomin® C5 active agents was investigated in two replicates of one batch of the coated formulation of the additive when added at 5 mg/kg to feed for poultry for fattening (based on maize and soyabean meal) and stored at 22°C in sealed light-protected and airtight bags for 3 months. The results showed no viability losses (< 0.5 log) at the end of the storage period.[37]

The stability of Biomin® C5 in water for drinking, was studied in two replicates of one batch of the non-coated formulation of the additive when supplemented at 1 g/L of tap water. Samples were stored at 30°C in in a sterile screwed cap glass flask for 48 h. Viability losses of < 0.5 log were observed.[38]

The capacity for homogeneous distribution of the additive in feed was studied in 10 subsamples of coated Biomin® C5 mixed with feed for chickens for fattening at an inclusion rate of 2.0×10^8 CFU/kg feed. The coefficient of variation ranged from 9.9% to 19.4%.[39]

3.1.5. Conditions of use

Biomin® C5 is intended to be used in feed and water for drinking for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species other than laying species at a proposed minimum concentration of 1×10^8 CFU/kg complete feed and 5×10^7 CFU/L in water for drinking.

The applicant indicated that the coated formulation of the additive is intended to be used in feed while the non-coated formulation in water for drinking.

3.2. Safety

The species *P. acidilactici*, *B. animalis*, *L. reuteri* and *L. salivarius* are considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to establishing safety for the target species (EFSA, 2007; EFSA BIOHAZ Panel, 2023). This approach requires the identification of the strain to be conclusively established and evidence that the strain does not show acquired resistance to antibiotics of human and veterinary importance. The identity of the four strains has been unambiguously established and the antibiotic resistance qualifications have been met. Consequently, the FEEDAP Panel considers that *P. acidilactici* DSM 33758, *B. animalis* DSM 16284, *L. reuteri* DSM 33751 and *L. salivarius* DSM 16351 meet the QPS requirements and thus, are presumed safe for the target species, consumers and the environment.

This presumption does not extend to the fifth strain (*E. faecium*). However, *E. faecium* DSM 33761 was unambiguously identified, found not to belong to the hospital-associated clade and not to express resistance to clinically relevant antibiotics. The metabolic end-products of the species are those typical of lactic acid bacteria and do not raise concerns. Moreover, *E. faecium* is a natural component of gut microbiota and its use as feed additive is unlikely to increase its presence in the wider environment. Therefore, the use of *E. faecium* DSM 33761 in animal nutrition is not expected to raise concerns for the target species, the consumers and the environment.

Considering the above and the fact that both preparations of the additive do not contain excipients of concern, the FEEDAP Panel concludes that Biomin® C5 is safe for target species, the consumers and the environment.

[35] Annex II_66 and Annex II_67.
[36] Annex II_68 and Annex II_69.
[37] Annex II_70.
[38] Annex II_71.
[39] Annex II_72.

3.2.1. Safety for the user

3.2.1.1. Effect on respiratory system

The highest measured dusting potential of the additive was to 2,795 and 2,250 mg/m^3 for the non-coated and coated formulations, respectively. Therefore, the FEEDAP Panel considered that the exposure through inhalation is likely. No studies on possible adverse effects of the additives on the respiratory system were submitted. Considering the proteinaceous nature of the active agents, both formulations of the additive should be considered as respiratory sensitisers.

3.2.1.2. Effect on eyes and skin

The applicant submitted three studies performed with the non-coated formulation of the additive.

The skin irritation potential of Biomin® C5 was investigated in an *in vitro* study performed according to OECD Testing Guideline (TG) 439. No conclusion could be drawn concerning the skin irritation potential of the additive since the test item was considered incompatible with the test method used.[40] The skin irritation potential of Biomin® C5 was further investigated in an *in vivo* study according to OECD TG 404, which showed that the test item is not a skin irritant (UN GHS classified as 'No Category').[41]

The eye irritation potential of Biomin® C5 was investigated in an *in vitro* study performed according to OECD TG 437. The results of the study indicated that, the test item cannot be classified in an UN GHS Category for eye damage with this study alone and is categorised as 'no stand-alone prediction can be made'.[42]

No data were provided on the coated formulation of the additive, but the applicant referred to the skin and eye irritancy studies[43] performed with Biomin® C3 and evaluated in a previous EFSA Opinion (EFSA FEEDAP Panel, 2012a, 2012b). Biomin® C3 is an additive containing *E. faecium* DSM 21913, *B. animalis* DSM 16284 and *L. salivarius* DSM 16351 which is a coated formulation similar to the coated one of Biomin® C5.[44] The studies performed with Biomin® C3 did not indicate a safety concern for the skin and eye irritation potential. Therefore, the FEEDAP Panel considered that the coated formulation of Biomin® C5 does not introduce additional risks compared to the non-coated formulation.

The FEEDAP Panel notes that the OECD TGs available at present are designed to assess the skin sensitisation potential of chemical substances only and that currently no validated assays for assessing the sensitisation potential of microorganisms are available. Therefore, no conclusions can be drawn on the skin sensitisation potential of the additive.

3.2.1.3. Conclusions on safety for the user

Considering the proteinaceous nature of the active agents, both formulations are considered respiratory sensitisers, but not skin irritants. The FEEDAP Panel is not in the position to conclude on the eye irritation and on dermal sensitisation potential of both forms of the additive.

3.3. Efficacy

The applicant provided four long-term trials in chickens for fattening to support the efficacy of the additive. However, in two of the trials,[45] the performance of the birds was below the standards of the breed under standard EU farming practices (ca. 70%) and showed high incidence of gut lesions, especially in one of the trials (incidence > 85%). Therefore, these two trials were not considered further as evidence of the efficacy of the additive.

The other two trials[46] were performed in the same trial site, with animals of the same genetic background obtained from the same hatchery, fed the same diet (same formulation and similar proximate analytical composition) and performed simultaneously for most of the experimental period (with a difference of 15 days between the reception of the two batches of birds). Therefore, the Panel

[40] Annex III_01.
[41] Annex III_02.
[42] Annex III_03.
[43] Annex III_SIN_Q5B and Annex III_SIN_Q5C.
[44] Biomin® C3 coating formulation: 4% w/w hydrogenated vegetable oil, 0.02% w/w hydroxypropyl methyl cellulose, 0.02% w/w methyl cellulose and 0.2% w/w xantham gum.
[45] Annex IV_01 to Annex_13 and Annex IV_19 to Annex_27.
[46] Annex IV_14 to Annex IV_18

considered these trials were not independent, and the data were pooled for the statistical analysis. The trial is described below, and results shown in Table 1.

A total of 1,560 one-day-old male chicks (Ross 308) were distributed in 52 pens of 30 animals each, and randomly allocated into two groups (26 replicates per treatment). Two basal diets (starter, from day 1 to 14; grower, from day 15 to 35) based on maize, soyabean meal and wheat, were either not supplemented (control) or supplemented with Biomin® C5 to provide a level of total bacteria of 1×10^8 CFU/kg feed. The level of the individual active agents in feed was analytically confirmed.[47] The experimental feeds were offered *ad libitum* in mash form for 35 days.

The animal health and mortality were daily checked, and the cause of death or reason for culling recorded. The chickens were individually weighed at the start of the trial (day 1). Thereafter, individual body weight (BW) and pen feed intake were recorded at days 15 and 35. The average daily gain, average daily feed intake and feed-to-gain ratio were calculated and corrected for mortality for the overall period.

The zootechnical performance data were analysed with a generalised linear model, including the diet as a fixed effect and the pen as the experimental unit.[48] Significance level was set at 0.05.

Table 1: Effects of the dietary supplementation with Biomin® C5 on the zootechnical performance of chickens for fattening

Biomin® C5 (CFU/kg complete feed)	Daily feed intake (g)	Initial body weight (g)	Final body weight (g)	Average daily weight gain (g)	Feed-to-gain ratio	Mortality and culling (%)
0	89.5	41	2,000[b]	55.1[b]	1.63[a]	2.56
1×10^8	89.2	41	2,100[a]	57.8[a]	1.54[b]	2.05

CFU: colony forming unit.
[a,b]: Mean values within a column with a different superscript are significantly different at $p < 0.05$.

The dietary supplementation of chickens for fattening with the additive at 1×10^8 CFU/kg complete feed for 35 days resulted in higher final BW and average daily weight gain, and better feed-to-gain ratio compared to the control group.

3.3.1. Conclusions on efficacy

Due to lack of sufficient data, the FEEDAP Panel is not in the position to conclude on the efficacy of the additive in chickens for fattening and, consequently, for chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species for fattening and reared for laying/breeding.

3.3.2. Compatibility with coccidiostats

The applicant provided *in vitro* studies to support the compatibility of *E. faecium* DSM 33761, *P. acidilactici* DSM 33758, *B. animalis* DSM 16284, *L. reuteri* DSM 33751 and *L. salivarius* DSM 16351 with nicarbazin, monensin sodium, robenidine hydrochloride, diclazuril, decoquinate, halofuginone, maduramicin ammonium and lasalocid A sodium.

The MIC values of these coccidiostats against the individual active agents were assessed *in vitro* using the broth microdilution methods. The MIC values for nicarbazin (> 2,000 mg/L), diclazuril (> 16 mg/kg), decoquinate (> 640 mg/kg) and halofuginone (> 48 mg/kg) were greater than four times the maximum authorised level of these coccidiostats in feed (125, 1, 40 and 3 mg/kg, respectively). The MIC values of the remaining coccidiostats, monensin sodium, robenidine hydrochloride, maduramicin ammonium and lasalocid A sodium were below four times their maximum authorised dose in feed (125, 36, 6 and 90 mg/kg, respectively), and additional *in vivo* studies would be required to support the compatibility of the additive' strains.

[47] Control diet – all values were on average below 1×10^5 CFU/kg feed. Biomin® C5 diets – Starter/Grower ($\times 10^7$ CFU/kg feed; average of analytical values of the two batches): *E. faecium,* 5.3/4.4; *P. acidilactici*, 3.6/3.8; *B. animalis*, 4.8/3.2; *L. reuteri*, 0.5/0.3; *L. salivarus*, 0.3/0.3.

[48] The interaction of the diet and batch of birds was checked; as no significant effect was found, it was not considered further in the analysis.

The results indicate that Biomin® C5 is compatible with nicarbazin, diclazuril, decoquinate and halofuginone. No conclusion can be reached on the compatibility with monensin sodium, robenidine hydrochloride, maduramicin ammonium and lasalocid A sodium.[49]

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[50] and Good Manufacturing Practice.

4. Conclusions

Both formulations of Biomin® C5 are considered to be safe for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species for fattening and reared for laying/breeding at the proposed conditions of use.

The use of the product as a feed additive is of no concern for the consumers and the environment.

Both formulations of Biomin® C5 should be considered respiratory sensitiser but they are not irritant to the skin. No conclusion can be made on the potential of the additive to be eye irritant or dermal sensitiser.

The FEEDAP Panel is not in the position to conclude on the efficacy of Biomin® C5 as a zootechnical additive for the target species at the proposed conditions of use.

Biomin® C5 is compatible with nicarbazin, diclazuril, decoquinate and halofuginone. No conclusions can be drawn on the compatibility of Biomin® C5 with monensin sodium, robenidine hydrochloride, maduramicin ammonium and lasalocid A sodium.

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[49] Annex II_73.

[50] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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[redacted]

Abbreviations

ADI	acceptable daily intake
ANI	average nucleotide identity
AMR	antimicrobial resistance
BW	body weight
CFU	colony forming unit
DSMZ	Deutsche Stammsammlung von Mikroorganismen und Zellkulturen
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
LOQ	limit of quantification
MIC	Minimum Inhibitory Concentration
NCBI	National Center for Biotechnology Information
OECD	Organisation for Economic Co-operation and Development
QPS	qualified presumption of safety
TG	Test Guideline
WGS	whole genome sequence