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SCIENTIFIC OPINION

Safety and efficacy of a feed additive consisting of muramidase produced with *Trichoderma reesei* DSM 32338 (Balancius™) for laying hens (DSM nutritional products)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of muramidase produced with *Trichoderma reesei* DSM 32338 (Balancius™) as a feed additive for laying hens. The additive is already authorised as a zootechnical additive (functional group: other zootechnical additives) for chickens, turkeys and minor poultry species for fattening or reared for breeding, and for weaned piglets. The enzyme is produced by fermentation with a genetically modified strain of *Trichoderma reesei;* viable cells of the production strain and its recombinant DNA were not detected in the additive. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the additive does not give rise to safety concerns regarding the genetic modification of the production strain. Based on the data available from a sub-chronic oral toxicity study, the Panel concluded that the additive is safe for laying hens at the maximum recommended level of 60,000 LSU(F) (muramidase activity units)/kg feed. The Panel also concluded that the additive is safe for the consumers and the environment. The liquid formulation of the additive is considered not irritant to the skin or eyes. The solid formulation of the additive is considered not irritant to the skin. The Panel cannot conclude on the potential of the additive (both formulations) to be a dermal sensitiser or on the potential of the solid formulation to be irritant to the eyes. Due to the proteinaceous nature, both forms of the additive should be considered respiratory sensitisers. The additive has the potential to be efficacious as a zootechnical additive for laying hens at 30,000 LSU(F)/kg feed.

KEYWORDS

Balancius™, efficacy, laying hens, muramidase, safety, zootechnical additives

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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 DSM Nutritional Products Ltd, represented in the EU by DSM Nutritional Products Sp. z o.o.,[2] for the new use of the additive consisting of muramidase produced with *Trichoderma reesei* DSM 32338 (Balancius™) as a feed additive for laying hens (category: zootechnical additive; functional group: other zootechnical additives).

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 21 October 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, consumer, user and the environment and on the efficacy of the feed additive consisting of muramidase produced with *Trichoderma reesei* DSM 32338, when used under the proposed conditions of use (see Section 3.1.3).

1.2 | Additional information

The additive is a preparation containing the enzyme muramidase produced by fermentation with a genetically modified strain of *Trichoderma reesei* (DSM 32338). It is authorised as a zootechnical additive for chickens and minor poultry species for fattening,[3] turkeys for fattening, chickens, turkeys and other poultry species reared for breeding[4] and weaned piglets[5] (4d16).

The FEEDAP Panel adopted three opinions on the product (EFSA FEEDAP Panel, 2018, 2019, 2021).

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[6] in support of the authorisation request for the use of muramidase produced with *Trichoderma reesei* DSM 32338 (Balancius™) as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 21 October 2022 to 21 January 2023; the comments received were considered for the assessment.

In accordance with Article 38 of Regulation (EC) No 178/2002[7] and taking into account the protection of confidential information and 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 concerning transparency and confidentiality,[8] a non-confidential version of the dossier has been published on Open.EFSA.[9]

[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]Tarczyńska 113, 96–320, Mszczonów, Poland.

[3]Commission Implementing Regulation (EU) 2019/805 of 17 May 2019 concerning the authorisation of a preparation of muramidase produced by Trichoderma reesei DSM 32338 as a feed additive for chickens for fattening and minor poultry species for fattening (holder of authorisation DSM Nutritional Products Ltd, represented in EU by DSM Nutritional Products Sp. Z o.o) C/2019/3643. OJ L 132, 20.5.2019, p. 33–35.

[4]Commission Implementing Regulation (EU) 2020/163 of 5 February 2020 concerning the authorisation of a preparation of muramidase (EC 3.2.1.17) (lysozyme) produced by Trichoderma reesei (DSM 32338) (Holder of authorisation DSM Nutritional Products Ltd, represented in EU by DSM Nutritional Products Sp. Z o.o), chickens reared for breeding, turkeys for fattening; turkeys reared for breeding; other poultry species reared for breeding. OJ L 34, 6.2.2020, p. 34.

[5]Commission Implementing Regulation (EU) 2021/1431 of 1 September 2021 concerning the authorisation of muramidase produced by Trichoderma reesei DSM 32338 as a feed additive for weaned piglets (holder of the authorisation DSM Nutritional Products Ltd, represented in the Union by DSM Nutritional Products Sp. z o.o.) (Text with EEA relevance).

[6]Dossier reference: FEED-2022-4411.

[7]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.

[8]Decision https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

[9]https://open.efsa.europa.eu/dossier/FEED-2022-4411

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 for the pre-submission phase and public consultations,[10] EFSA carried out a public consultation on the non-confidential version of the application from 16 November to 7 December 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 to deliver the present output.

The European Union Reference Laboratory considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the active substance in animal feed are valid and applicable to the current application.[11]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of muramidase produced with *Trichoderma reesei* DSM 32338 (Balancius™) is in line with the principles laid down in Regulation (EC) No 429/2008[12] and the relevant guidance documents: 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, 2018b), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (2018c) and Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023).

3 | ASSESSMENT

The present opinion deals with the assessment of the safety and efficacy of the enzyme preparation containing muramidase produced by fermentation with *Trichoderma reesei* DSM 32338, referred here below with its trade name, Balancius™, as a zootechnical feed additive (functional group: other zootechnical additives, support the digestive function) for laying hens.

3.1 | Characterisation

3.1.1 | Characterisation of the production organism

Balancius™ contains muramidase (Enzyme Commission number 3.2.1.17, lysozyme or N-acetylmuramidase) produced by fermentation with a genetically modified strain of *T. reesei* deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) with the accession number DSM 32338.[13]

The taxonomic identification of the production strain as *T. reesei* was confirmed by whole genome sequencing-based analysis.[14] [redacted]. Analysis with USEARCH algorithm further showed that 92%–99% of the total number of genes are orthologous among the genomes of DSM 32338 and *T. reesei* QM6a and *T. reesei* RUT-C30.

The production strain was derived from *T. reesei* RUT-C30, [redacted].

The genetic modification of the production strain was assessed in a previous assessment and the Panel concluded that the genetic modification does not raise any safety concerns (EFSA FEEDAP Panel, 2018a). The data submitted for the current authorisation are not different from the previously evaluated one, and therefore, the same conclusions apply.

Some *Trichoderma* species are known to be capable of producing a variety of mycotoxins and antifungal metabolites. In the context of the current assessment, the applicant referred to a bioinformatic analysis conducted on the genome of a precursor strain (*T. reesei* BTR213) that was assessed for a previous opinion (EFSA FEEDAP Panel, 2018a).[15] The analysis showed that no relevant genes involved in the production of kojic acid and trichodermin are harboured by the precursor strain of DSM 32338. This is in agreement with previous reports finding *T. reesei* unable to produce mycotoxins (EFSA, 2007; EFSA BIOHAZ Panel, 2020; Frisvad et al., 2018). However, this species is known to produce peptaibols, such as paracelsin A,

[10]Decision https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

[11]The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/document/download/6d48b339-eaba-4180-a124-4b030f4603ca_en?filename=finrep-fad-2017-0046-muramidase.pdf

[12]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.

[13]RFI-2 Annex 1_Deposit confirmation from DSMZ _DSM_32338.

[14]Annex 2.1.17 DSM 32338 GMM dossier and RFI-2 Annex 2_ EFSA Q 2 ANI explanation for DSM 32338.

[15]RFI III.2024 - Annex 1.A.

C and D (Frisvad et al., 2018); therefore, the presence of paracelsins was analysed in five batches of both final formulations of the additive and showed in all cases results below the limit of detection (LOD) of the analytical method (< [redacted]).[16]

3.1.2 | Characterisation of the additive

The additive Balancius™ is available in two different formulations: solid (Balancius™ GT) and liquid (Balancius™ L), both with a guaranteed minimum activity of 60,000 LSU(F)[17] per gram of product.

The Panel has already assessed data on the same additive regarding the manufacturing process, physico-chemical properties, stability and homogeneity and the presence of DNA of the production strain in the additive (EFSA FEEDAP Panel, 2018, 2019, 2021). Since the manufacturing process and the composition of the additive have not been modified, the data reported in previous opinions in regard to the composition and characterisation of the additive are still valid. However, the applicant submitted updated data on the batch-to-batch variation of five batches, and on the presence of impurities and viable cells of the production strain.

Analytical data to confirm the specifications were provided for five batches of each formulation of the additive, showing the following average values: 77,400 LSU(F)/g (range 74,600–80,600) and 90,900 LSU(F)/g (range 84,700–96,900) for the solid and liquid formulation, respectively.[18]

The same five batches from both formulations of the additive were analysed for impurities. Cadmium, lead, mercury and arsenic concentrations showed values below the LOD of the analytical method in all batches, except for arsenic in the solid formulation where concentrations were on average 0.45 mg/kg (range 0.43–0.49).[19]

Microbiological contamination in both formulations of the additive was analysed in the same five batches by the determination of total viable counts (< 100 colony forming units (CFU)/g), yeasts and filamentous fungi (< 10 CFU/g), coliforms (< 10 CFU/g), *Enterobacteriaceae* (< 10 CFU/g), *Bacillus cereus* (< 10 CFU/g), *Escherichia coli* and *Salmonella* spp. (not detected in 25 g). Presumptive *B. cereus* (90 CFU/g) and filamentous fungi (20 CFU/g) were detected in one batch of the liquid formulation.[20]

The FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

The presence of viable cells of the production strain was investigated in three batches of the intermediate concentrate ([redacted]) that is used to formulate the two final formulations of the additive, each analysed in triplicate.[21] [redacted] No growth was detected.

No new data were submitted to check the presence of DNA from the production strain. The Panel considers that the data submitted in the context of a previous application, which showed no detection with a LOD of 10 ng/mL, are still valid and applicable for the current submission (EFSA FEEDAP Panel, 2021).

3.1.3 | Conditions of use

The additive is intended for use in feed for laying hens with a minimum recommended enzyme activity of 30,000 LSU(F)/kg feed and a recommended level between 30,000 and 60,000 LSU(F)/kg feed.

3.2 | Safety

3.2.1 | Safety of the production microorganism

The assessment of the genetic modification of the production strain, *T. reesei* DSM 32338, was performed in a previous opinion and the Panel concluded that the genetic modification does not raise any safety concerns (EFSA FEEDAP Panel, 2018a). The production strain has not been subject to any further genetic modification and no new information has been made available that would lead the Panel to reconsider its previous conclusion. Moreover, viable cells and DNA of the production

[16]RFI-1 Annex 1.5 Purity analyses Balancius GT_EFSA notification 2023–00017944, RFI-1 Annex 1.6 Purity analyses Balancius L EFSA notification 2023–00017615 and RFI III.2024 – Annex 1.D. Liquid chromatography coupled to a Orbitrap mass spectrometer (LC/MS).

[17]LSU(F) is defined as the amount of enzyme that increases the fluorescence of 12.5 μg/mL fluorescein-labelled peptidoglycan per minute at pH 6.0 and 30°C by a value that corresponds to the fluorescence of approximately 0.06 nmol fluorescein isothiocyanate isomer.

[18]RFI-1 Annex 1.1 Balancius GT B2B EFSA notification no 2023–00018059 and RFI-1 Annex 1.2 Balancius L B2B Efsa Notification 2023–00017614.

[19]RFI-1 Annex 1.3 Balancius GT heavy metals EFSA notification 2023–00017945 and RFI-1 Annex 1.4 Balancius L Heavy metals EFSA notification no 2023–00017612; [redacted].

[20]RFI-1 Annex 1.5 Purity analyses Balancius GT_EFSA notification 2023–00017944 and RFI-1 Annex 1.6 Purity analyses Balancius L EFSA notification 2023–00017615.

[21]RFI-2 Annex 3 New Annex E1 Abs of GMM.

strain were not detected in the additive. Therefore, the use of *T. reesei* DSM 32338 to produce the muramidase under assessment does not raise any safety concerns as regards the genetically modified production strain.

3.2.2 | Toxicological studies

In the context of the previous assessment, the applicant submitted two genotoxicity studies (a bacterial reverse mutation test and an in vitro chromosomal aberration test) and a sub-chronic oral toxicity study in rats (EFSA FEEDAP Panel, 2018a). The FEEDAP Panel concluded that, based on the experimental conditions applied in the bacterial reverse mutation test and the sub-chronic oral toxicity study, the intermediate fermentation filtrate did not show any mutagenic potential and the no observed adverse effect level (NOAEL) was set at the maximum concentration tested (384,616 LSU(F) per kg body weight). The Panel considers that these conclusions can be retained for the current assessment. However, based on the Guidance on aneugenicity assessment (EFSA Scientific Committee, 2021), the Panel requested a further investigation on the potential to induce numerical chromosome aberration, not properly assessed by the previously submitted in vitro chromosomal aberration test.

In the current application, the applicant submitted an in vitro mammalian cell micronucleus test in human peripheral blood lymphocytes to evaluate the potential of an intermediate enzyme concentrate (10.8% total organic solids [TOS]; 36,700 LSU(F)/g) to induce chromosomal damage.[22] The test was performed following Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 487 and claimed to be compliant with good laboratory practices. Based on the results of a preliminary cytotoxicity assay, muramidase (lysozyme) was tested by applying a short treatment (3 + 17 h of recovery) in the presence and absence of metabolic activation at 3000, 4000 and 5000 μg TOS/mL. A continuous treatment (20 h) was also conducted in the absence of metabolic activation testing Lysozyme at 100, 1000 and 3000 μg TOS/mL. The test item did not induce a significant increase in the frequency of binucleated micronucleated cells compared to the vehicle control cultures. Therefore, the FEEDAP Panel concludes that the test item did not induce structural and numerical chromosome aberrations in vitro in human peripheral blood lymphocytes under the experimental conditions employed in this study.

Based on the genotoxicity trials submitted, the FEEDAP Panel concludes that the additive shows no genotoxic potential under the tested conditions.

3.2.3 | Safety for the target species

The results of a sub-chronic oral toxicity study in rats, already assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2018a), were used to support the safety of the additive for the laying hens. In this study, a NOAEL of 384,616 LSU(F)/kg body weight and day was identified in rats. Using this NOAEL and applying the procedure detailed in the Guidance on the safety for the target species (EFSA FEEDAP Panel, 2017b), the maximum safe level for laying hens is calculated to be 63,861 LSU(F)/kg feed. The Panel concludes that the additive is safe for laying hens at the highest recommended level of 60,000 LSU(F)/kg.

3.2.4 | Safety for the consumers

The results obtained in the genotoxicity and sub-chronic oral toxicity studies performed with the test item did not indicate safety concerns. The proposed use of the additive in the feed of laying hens would not introduce risks not already evaluated in the previous opinion. Therefore, the FEEDAP Panel concludes that the use of the additive under the proposed conditions of use is safe for the consumers.

3.2.5 | Safety for the user

3.2.5.1 | *Effect on the respiratory system*

No specific studies were provided by the applicant regarding the effects of the additive on the respiratory system. The dusting potential of the solid formulation is negligible, and the exposure of the user is unlikely (EFSA FEEDAP Panel, 2018a). Owing to the proteinaceous nature of the active substance, the additive is considered a respiratory sensitiser.

3.2.5.2 | *Effects on the skin and eyes*

The skin irritation potential of the solid and liquid formulations of the additive was assessed by the in vitro EpiDerm™ reconstructed human epidermis according to OECD TG 439.[23] Based on the results obtained, both solid and liquid formulations are classified as non-irritant in accordance with the UN GHS 'No Category'.

[22]RFI-2 Annex 4_Balancius_In vitro micronucleus study.

[23]Annex 6.3 Skin irritation study Balancius Solid and Annex 6.4 Skin irritation study Balancius liquid.

The eye irritation potential of the solid and liquid formulations was assessed by Bovine Corneal Opacity and Permeability Assay according to OECD TG 437.[24] Based on the results obtained, the liquid formulation is classified as non-irritant in accordance with the UN GHS 'No Category'. As regards the solid formulation, the results do not allow a stand-alone prediction for 'No Category' nor 'Serious Eye irritant Category 1', as the In Vitro Irritancy Score was between 3 and 55 for both cases. Consequently, the FEEDAP Panel could not conclude on the eye irritation potential of the solid formulation.

No specific studies investigating the skin sensitisation potential of the additive were submitted.

3.2.5.3 | *Conclusions on safety for the user*

The liquid formulation of the additive is considered not irritant to the skin and eyes. The solid formulation of the additive is considered not irritant to the skin. The Panel cannot conclude on the potential of the additive (both formulations) to be a dermal sensitiser, or on the potential of the solid formulation to be irritant to the eyes. Due to the proteinaceous nature of the active substance, both forms of the additive should be considered respiratory sensitisers.

3.2.6 | Safety for the environment

In a previous opinion, the Panel concluded that the use of the product as a feed additive raises no concerns for the environment (EFSA FEEDAP Panel, 2018a). The FEEDAP Panel is not aware of any new information that would lead to reconsidering the conclusions drawn previously for the environment and considers that the extension of use to the new species for which the application is made would not have an impact on the safety aspects already evaluated. Therefore, the use of the additive under the proposed conditions of use is considered safe for the environment.

3.3 | Efficacy

3.3.1 | Efficacy in laying hens

Three long-term trials were performed aiming at assessing the effect of muramidase on the zootechnical performance of laying hens. The three trials shared a common design, a summary of which is shown in Table 1. The results of the effect of the additive on the zootechnical performance parameters of laying hens are shown in Table 2.

TABLE 1 Trial design and use level in the efficacy trials performed in laying hens.

Trial	Total no of animals (animals × replicate) replicates × treatment	Breed (duration)	Composition feed (form)	Groups (LSU(F)/kg complete feed)	
				Intended	Analysed
1[25]	210 (5) 21	ISA Brown (140 days)	Maize, wheat, soybean meal (mash)	0 30,000	< 2000 29,000
2[26]	576 (8) 24	Hy-Line Brown (140 days)	Maize, wheat, soybean meal (mash)	0 15,000 30,000	< 2000 16,750 29,733
3[27]	945 (15) 21	Lohmann Brown (84 days)	Maize, barley and soybean meal (mash)	0 15,000 30,000	< 2000 12,800 30,300

Abbreviation: LSU(F), muramidase enzymatic activity units.

In the three trials, laying hens of different genetic backgrounds were distributed in enriched cages and randomly allocated to two (Trial 1) or three (Trials 2 and 3) experimental groups. For that purpose, the basal diets were either not supplemented (control) or supplemented with Balancius™ to provide 30,000 LSU(F)/kg complete feed (trial 1), or 15,000 and 30,000 LSU(F)/kg complete feed (trials 2 and 3). The analysed enzyme activities of the feeds are shown in Table 1. In all trials, the mortality and general health were monitored daily throughout the experimental period, and the most likely cause of death/culling was recorded. Animals were weighed at the start and end of the trial.

In Trial 1, egg production per cage was recorded daily, and feed consumption and egg mass every 28 days. The average daily feed intake (ADFI), laying rate and feed-to-egg mass ratio were calculated for the whole experimental period (22–42 weeks of age). At Weeks 4, 8, 12, 16 and 20 of the study, all eggs laid in 1 day were collected, individually weighed

[24] Annex 6.1 Eye irritation study report_Balancius solid and Annex 6.2 Eye irritation study report_Balancius liquid.
[25] Annex 4.7 D2290820 IMASDE Balancius layers.
[26] Annex 4.8 RD-00066264_G-236 IRTA.
[27] Annex 4.10 RD-00066263_FK171120 Balancius in layers SRUC.

and classified according to commercial standards (S/M/L/XL). The experimental data were analysed with a generalised linear model with the treatment and the block (location in the room) as fixed effects. The significance level was set at 0.05.

In Trial 2, egg production per cage was recorded every 2 days, egg quality characteristics (normal, broken, dirty, shell-less and misshapen), feed consumption and egg mass were recorded every 28 days. The ADFI, laying rate and feed-to-egg mass ratio were calculated for the whole experimental period (22–42 weeks of age). The experimental data were analysed with a two-way ANOVA with the treatment and the block (location in the house) as fixed effects. Means of different treatment groups were compared using Tukey's test. The significance level was set at 0.05.

In Trial 3, egg production per cage and egg mass were recorded daily, and feed consumption was every 14 days. ADFI, laying rate and feed-to-egg mass ratio were calculated for the whole experimental period (22–34 weeks of age). The experimental data were analysed with a two-way ANOVA with the treatment and the block (location in the house) as fixed effects. Means of different groups were compared using Tukey's test. The significance level was set at 0.05.

Average mortality and culling rates in all trials were in line with commercial standards (1.42%, 3.8% and 0.5% for trials 1, 2 and 3, respectively) and with no differences between groups. In comparison with the control group, hens receiving diets supplemented with the minimum recommended level of Balancius™ (30,000 LSU(F)/kg feed) showed higher laying rate in the three trials and improved feed-to-egg mass ratio in Trials 1 and 2. The inclusion of the additive in the feed of laying hens showed no detrimental effect on the egg quality in any of the trials.

TABLE 2 Effects of Balancius™ on the zootechnical performance of laying hens.

Trial	Groups (LSU(F)/kg feed)	Daily feed intake (g)	Final body weight (g)	Laying rate (%)	Egg weight (g)	Egg mass (g/hen per day)	Feed-to-egg mass	Mortality and culling (%)
1	0	124.5	1825b	90.9^{b}	60.6	55.0^{b}	2.27^{a}	0.9
	30,000	124.1	1912a	95.1^{a}	60.3	57.3^{a}	2.17^{b}	1.9
2	0	105.9	1882^{b}	92.9^{b}	61.3	56.9^{b}	1.86^{a}	4.2
	15,000	106.7	1947^{a}	94.1^{ab}	61.4	57.7^{ab}	1.85^{ab}	2.6
	30,000	105.9	1922^{ab}	95.2^{a}	62.1	59.1^{a}	1.79^{b}	4.7
3	0	120.3	1993	96.4^{b}	59.7	57.5^{b}	2.12	0
	15,000	119.5	1991	97.1^{ab}	59.7	57.9^{ab}	2.09	1.3
	30,000	120.7	2008	97.9^{a}	59.9	58.6^{a}	2.08	0.3

^{a,b}Mean values within a trial and within a column with a different superscript are significantly different $p < 0.05$.

3.3.2 | Conclusions on efficacy

The additive when added to feed at 30,000 LSU(F)/kg feed has the potential to be efficacious in laying hen.

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[28] and Good Manufacturing Practice.

4 | CONCLUSIONS

The production strain and its recombinant DNA were not detected in the additive. The additive does not give rise to safety concerns regarding the genetic modification of the production strain.

The FEEDAP Panel concludes that the additive is safe for laying hens at the maximum recommended level of 60,000 LSU(F)/kg feed.

The use of Balancius™ as a feed additive at the proposed conditions of use is considered safe for the consumers and the environment.

The liquid formulation of the additive is considered not irritant to the skin or eyes. The solid formulation of the additive is considered not irritant to the skin. The Panel cannot conclude on the potential of the additive (both formulations) to be a dermal sensitiser or on the potential of the solid formulation to be irritant to the eyes. Due to the proteinaceous nature of the active substance, both forms of the additive should be considered respiratory sensitisers.

The additive is considered to be efficacious as a zootechnical additive for laying hens at 30,000 LSU(F)/kg complete feed.

[28]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.

ABBREVIATIONS

ADFI	average daily feed intake
ANI	average nucleotide identity
CFU	colony forming unit
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
TG	Test guideline
WGS	Whole genome sequence
TOS	Total organic solids

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CONFLICT OF INTEREST

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REFERENCES

EFSA (European Food Safety Authority). (2007). Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. *EFSA Journal*, 5(12), 587. https://doi.org/10.2903/j.efsa.2007.587

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (2020). Scientific Opinion on the update of the list of QPS recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019). *EFSA Journal*, 18(2), e05966. https://doi.org/10.2903/j.efsa.2020.5966

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Innocenti, M. L. (2017a). Guidance on the assessment of the safety of feed additives for the consumer. *EFSA Journal*, *15*(10), 5022. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Innocenti, M. L. (2017b). Guidance on the identity, characterisation and conditions of use of feed additives. *EFSA Journal*, *15*(10), 5023. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Martino, L. (2017c). Guidance on the assessment of the safety of feed additives for the target species. *EFSA Journal*, *15*(10), 5021. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., Lopez-Alonso, M., Lopez Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., Villa, R. E., … Cocconcelli, P. S. (2018a). Scientific opinion on the safety and efficacy of muramidase from *Trichoderma reesei* DSM 32338 as a feed additive for chickens for fattening and minor poultry species. *EFSA Journal*, *16*(7), 5342. https://doi.org/10.2903/j.efsa.2018.5342

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Martino, L. (2018b). Guidance on the assessment of the efficacy of feed additives. *EFSA Journal*, *16*(5), 5274. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Galobart, J. (2018c). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*, *16*(3), 5206. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Dusemund, B., Kouba, M., Kos Durjava, M., Lopez-Alonso, M., Lopez Puente, S., Marcon, F., Mayo, B., Pechova, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Anguita, M., … Holczknecht, O. (2019). Scientific opinion on the safety and efficacy of muramidase from *Trichoderma reesei* DSM 32338 as a feed additive for turkeys for fattening, turkeys reared for breeding, chickens reared for breeding and other poultry species reared for breeding. *EFSA Journal*, *17*(5), 5686. https://doi.org/10.2903/j.efsa.2019.5686

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances usedin Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Dusemund, B., Fasmon Durjava, M., Kouba, M., Lopez-Alonso, M., Lopez Puente, S., Marcon, F., Mayo, B., Pechova, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Cocconcelli, P. S., … Anguita, M. (2021). Scientific opinion on the safety and efficacy of the additive consisting of muramidase produced by *Trichoderma reesei* DSM 32338 (Balancius™) for use in weaned piglets (DSM nutritional products ltd). *EFSA Journal*, *19*(3), 6452. https://doi.org/10.2903/j.efsa.2021.6452

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Durjava, M., Dusemund, B., Kouba, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Villa, R. E., Woutersen, R., Brantom, P., Chesson, A., … Galobart, J. (2023). Guidance on the assessment of the safety of feed additives for the users. *EFSA Journal*, *21*(12), e8469. https://doi.org/10.2903/j.efsa.2023.8469

EFSA Scientific Committee, More, S. J., Bampidis, V., Bragard, C., Halldorsson, T. I., Hernández-Jerez, A. F., Hougaard Bennekou, S., Koutsoumanis, K., Lambré, C., Machera, K., Naegeli, H., Nielsen, S. S., Schlatter, J., Schrenk, D., Turck, D., Younes, M., Aquilina, G., Bignami, M., Bolognesi, C., … Benford, D. (2021). Scientific opinion on the guidance on aneugenicity assessment. *EFSA Journal*, *19*(8), 6770. https://doi.org/10.2903/j.efsa.2021.6770

Frisvad, J. C., Moller, L. L. H., Larsen, T. O., Kumar, R., & Arnau, J. (2018). Safety of the fungal workhorses of industrial biotechnology: Update on the mycotoxin and secondary metabolite potential of *aspergillus Niger*, *aspergillus oryzae*, and *Trichoderma reesei*. *Applied Microbiology and Biotechnology*, *102*, 9481–9515.

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