Assessment : Assessment of the safety of the additive consisting of muramidase produced by *Trichoderma reesei* DSM 32338 (Balancius) for use as a feed additive for weaned piglets (DSM Nutritional Products Ltd)

Reference Number RP1259

Risk Assessment Unit
Science, Evidence and Research Division, FSA

Risk Assessment Team
Science Division, FSS

29th August 2023

Contents

1.Executive summary 2
2.Background and purpose of review 2
3.Details of the EFSA assessment 3
3.1 Methodology applied in the EFSA opinion 3
3.2 Source/organism 3
3.3 Genetic modification step 3
3.4 Specification 4
3.5 Exposure assessment 4
3.6 Toxicological data 4
4. EFSA assessment and conclusions 5
5. Caveats and uncertainties 5
6. FSA Conclusion on reliability and applicability 5
6.1 Analytical Method Review 6
7. Outcome of assessment 6
8. References 6

1.Executive summary

The Food Standards Agency (FSA) and Food Standards Scotland (FSS) have undertaken an assessment of application RP1259 for the extension of use of muramidase produced by *Trichoderma reesei* DSM 32338 (Balancius) (minimum activity of 60,000 LSU(F)/g[1]), from DSM Nutritional Products Ltd. Switzerland, Wurmisweg 576, CH-4303, Kaiseraugst, Switzerland (category: zootechnical additives; functional group: other zootechnical additives (improvement of the feed to gain ratio)). The application is to extend the use to weaned piglets.

A feed additive application has been received by Great Britain (GB) where EFSA, prior to the end of the transition period, evaluated an application for the product. FSA/FSS have reviewed the EFSA opinion (EFSA Journal 2021;19(3):6452, 9 pp.) and confirm that it is adequate and relevant for GB risk analysis and used this to form the basis of the GB opinion.

The FSA/FSS risk assessors concluded that the EFSA opinion is adequate and relevant for GB risk analysis. Therefore, the use of Balancius, 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.

There are no specific conditions or restrictions in relation to handling, labelling, post-market monitoring requirements and use of this additive as described in this application. Maximum Residue Limits (MRLs) are not required for this additive.

2.Background and purpose of review

EFSA Journal 2021;19(3):6452 **Question number**: EFSA-Q-2020-00199

In accordance with Retained EU Regulation 1831/2003 on feed additives, the application RP1259 for the extension of use of muramidase produced by *Trichoderma reesei* DSM 32338 (Balancius) (minimum of 50,000 LSU(F)/kg feed), from DSM Nutritional Products Ltd. has been submitted for authorisation in GB.

Whilst it was a Member State of the EU, UK accepted the assessments of EFSA in respect of authorisations for regulated food and feed products. Since the end of the transition period, FSA/FSS has adopted equivalent technical guidance and quality assurance processes to be able to undertake GB risk assessments for regulated product applications.

Where EFSA, prior to the end of the transition period, evaluated an application for the product for which an application is now made to GB, FSA/FSS has decided to make use of the EFSA risk assessment, where this is appropriate, in forming its

[1] 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.

opinion. Therefore, FSA/FSS risk assessors have reviewed the EFSA opinion[2] for the application below in the context of intended GB use and have concluded that the intended uses are safe.

In reviewing the output of the EFSA risk assessment the reviewers have verified that the standard approach as outlined in the relevant guidance[3] has been followed and the arguments made are consistent with the data summarised in the opinion. Consideration has been given to the processes undertaken to ensure the 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 the EFSA scientific opinion is adequate also for GB risk analysis. Therefore, a full risk assessment has not been performed by FSA/FSS.

3.Details of the EFSA assessment

3.1 Methodology applied in the EFSA opinion

EFSA FEEDAP guidance: Guidance on the identity, characterisation and conditions of use of feed additives (**2017a**), Guidance on the assessment of the safety of feed additives for the target species (**2017b**), Guidance on the assessment of the safety of feed additives for the consumer (**2017c**), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (**2018a**), Guidance on the assessment of the safety of feed additives for the environment (**2019**) and principles in Regulation (EC) No. 429/2008.

3.2 Source/organism

The additive contains muramidase, which is produced by a genetically modified strain of *T. reesei* deposited with Sammlung von Mikroorganismen und Zellkulturen with the accession number DSM 32338.

The characterisation of the additive, and the production strain, were undertaken in a previous EFSA opinion (EFSA, 2018b). Solid and liquid forms of the additive both contain a minimum activity of 60,000 LSU(F) per gram. The additive also contains endo-1,4-β-glucanase, endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase activity as side activities. The parental organism was considered a safe production organism (EFSA, 2018b).

3.3 Genetic modification step

The assessment of the genetic modification of the production strain was performed in a previous evaluation (EFSA, 2018b), and has not been modified. EFSA (2018b) concluded that the introduced sequences did not give rise to safety concerns. There are no antimicrobial resistance genes in the production strain remaining from the genetic modification process. No viable cells and no DNA of the production strain

[2] EFSA (2021). 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, 9 pp. https://doi.org/10.2903/j.efsa.2021.6452

[3] See reference list for the full set of guidance applied.

were detected in the additive. EFSA (2018b) concluded that the product does not give rise to any safety concerns with regard to the genetic modification.

3.4 Specification

As noted above, the additive is available in both solid and liquid forms and has a guaranteed minimum activity of 60,000 LSU(F) per gram of product. In the context of the current application, the applicant provided new data on the shelf-life of the product and on the presence of DNA of the production strain in the additive, which were evaluated by EFSA.

Shelf-life was studied in samples of at least three batches of each formulation, stored at 10˚C, 25˚C and 35˚C for up to 24 months. Sub-samples of the same batches were also stored at -18˚C for the same periods of time for comparison. The solid formulation showed recoveries of activity of 94%, 86% and 54% after 24 months at 10˚C, 25˚C and 35˚C, respectively, thus there was compliance with the specification only up to 25˚C. The liquid formulation showed recoveries of activity of 86%, 63% and 36% after 24 months at 10˚C, 25˚C and 35˚C, respectively, thus there was compliance with the specification only up to 10˚C.

Some of the information on the assessment of the presence of DNA of the production strain in the additive has been redacted due to commercial confidentiality. However, EFSA has stated that no DNA from the production strain was identified in the samples analysed.

3.5 Exposure assessment

Not relevant.

There are no residues and therefore there is no dietary exposure assessment for consumers. EFSA previously assessed the safety of the genetic modification of the production strain and the additive for food consumers and concluded that the use of this feed additive poses no concerns for consumer safety (EFSA, 2018b). In this opinion, EFSA has concluded that the extension of use to piglets has no impact on the safety aspects already assessed.

3.6 Toxicological data

This application is for the extension of use of the additive consisting of muramidase produced by *Trichoderma reesei* DSM 32338 (Balancius) for use in weaned piglets. No new toxicological data have been submitted. EFSA previously assessed the safety of the genetic modification of the production strain and the additive for food consumers and concluded that the use of this feed additive poses no concerns for consumer safety (EFSA, 2018b). Regarding the safety for the user EFSA could not conclude on the potential of the additive for skin/eye irritancy and skin sensitisation, but concluded that it should be considered a respiratory sensitiser (EFSA, 2018b). EFSA was not aware of any new information that would lead it to reconsider the conclusions drawn previously, and considered that the extension of use to the new species, weaned piglets, for which this application is made would not have any impact on the safety aspects already evaluated. However, the safety for the new target species needed to be addressed.

EFSA used the existing subchronic oral toxicity study in rats, previously evaluated by EFSA (2018b), to estimate the maximum safe level of the additive in the feed of the

weaning piglets. The no observed adverse effect level (NOAEL) from the study was 384,616 LSU(F)/kg bodyweight per day. EFSA has stated that it applied the procedure in the guidance on the assessment of the safety of feed additives for the target species (2017b) to conclude that the maximum safe level in the diet of weaned piglets is 76,923 LSU(F)/kg feed. FSA/FSS has checked the calculations. As per the EFSA guidance (2017b) a margin of safety of 100 is applied, and therefore the level of intake without appreciable risk to the weaned piglets is 3,846.16 LSU(F)/kg bodyweight per day. This is converted to a concentration in the feed by dividing it by a default value for the daily feed intake of a piglet of 44 g dry matter (DM) per kg bodyweight and multiplying the resulting figure by 1000 to express it per kg of feed. The resulting concentration is 87,413 LSU(F)/kg DM feed. This is then multiplied by 0.88 to convert from a DM basis to an as-is basis, assuming a moisture content of 12% for the feed. The resulting concentration is 76,923 LSU(F)/kg feed, as calculated by EFSA.

The maximum recommended level of the additive in the feed of the piglets is 65,000 LSU(F)/kg and EFSA has therefore concluded that the additive is safe for piglets.

4. EFSA assessment and conclusions

Muramidase produced by *Trichoderma reesei* DSM 32338 (Balancius) is safe for weaned piglets up to the maximum recommended level in feed of 65,000 LSU(F)/kg.

Its use as a feed additive for weaned piglets is safe for consumers and the environment. Regarding risks to workers, the additive should be considered a respiratory sensitiser; however, EFSA could not conclude on the potential of the additive for skin/eye irritancy and skin sensitisation.

5. Caveats and uncertainties

There are no further caveats or uncertainties to highlight.

6. FSA Conclusion on reliability and applicability

The application has been assessed in line with the applicable guidance. The conclusions for this extension of use are largely based on a previous opinion in 2018 (EFSA, 2018b) on both the feed additive and the genetic modification. EFSA was not aware of any new information that would lead it to reconsider the conclusions drawn previously and considered that the extension of use to the new species (weaned piglets) for which this application is made would not have any impact on the safety aspects already evaluated. However, EFSA has considered the safety for the new target species, weaned piglets. EFSA has used existing toxicological information, previously assessed in 2018 (EFSA, 2018b), to conclude that the additive is safe for weaned piglets up to the maximum recommended level in feed of 65,000 LSU(F)/kg. FSA/FSS agrees with this assessment.

6.1 Analytical Method Review

FSA/FSS accepts the EURL analytical method evaluation report.[4] FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

7. Outcome of assessment

FSA/FSS has reviewed the EFSA opinion and consider it adequate and relevant for GB risk analysis. Therefore, the opinion was used to form the basis of the GB opinion.

FSA/FSS had access to all supporting documentation that was provided to the EFSA Panel by the applicant, and subsequently used to form the EFSA opinion. FSA/FSS agree with the safety conclusions outlined in the EFSA opinion.

The FSA/FSS opinion is that muramidase produced by *Trichoderma reesei* DSM 32338 (Balancius), 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.

8. References

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2017a. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 12 pp. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2017b. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2017c. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pp. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2018a. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2018b. Scientific Opinion on the safety and efficacy of muramidase from *Trichoderma reesei* DSM 32338 as a feed additive for chickens for fattening

[4] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/system/files/2018-03/finrep-fad-2017-0046-muramidase.pdf

and minor poultry species. EFSA Journal 2018;16(7):5342, 16 pp. https://doi.org/10.2903/j.efsa.2018.5342

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2019. Guidance on the assessment of the safety of feed additives for the environment. EFSA Journal 2019;17(4):5648, 78 pp. https://doi.org/10.2903/j.efsa.2019.5648

EFSA (2021). 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, 9 pp. https://doi.org/10.2903/j.efsa.2021.6452