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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

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Abstract

Following a request from the European Commission, the 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 muramidase produced by *Trichoderma reesei* DSM 32338. The additive is considered safe for turkeys for fattening, turkeys reared for breeding, chickens reared for laying/breeding and other poultry species reared for breeding up to the maximum recommended dose of 45,000 LSU(F)/kg feed. The additive is considered safe for the consumer and the environment. No conclusions can be reached on the potential of the additive for skin/eye irritancy and skin sensitisation. The additive should be considered a potential respiratory sensitiser. The additive has the potential to be efficacious as a zootechnical additive in turkeys for fattening, turkeys reared for breeding, chickens reared for laying/breeding and other poultry species reared for breeding when added to feed at 25,000 LSU(F)/kg feed.

Keywords: zootechnical additives, other zootechnical additives, muramidase, safety, efficacy, turkeys for fattening, poultry reared for breeding

Requestor: European Commission

Question number: EFSA-Q-2018-00952

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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 a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from DSM Nutritional Products Ltd., Switzerland[2] for authorisation of the product muramidase produced by *Trichoderma reesei* DSM 32338, when used as a feed additive for turkeys for fattening, turkeys reared for breeding, chickens reared for breeding and other poultry species reared for breeding (category: zootechnical additives; 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). The particulars and documents in support of the application were considered valid by EFSA as of 16 January 2019.

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 product muramidase produced by *Trichoderma reesei* DSM 32338, when used under the proposed conditions of use (see Section 3.1.1).

1.2. Additional information

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of muramidase produced by *Trichoderma reesei* DSM 32338 when used as a feed additive for chickens for fattening and minor poultry species (EFSA FEEDAP Panel, 2018a). The product is not authorised in the EU as a feed additive.

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 muramidase produced by *Trichoderma reesei* DSM 32338 as a feed additive.

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 (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of muramidase produced by *Trichoderma reesei* DSM 32338 is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b).

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] DSM Nutritional Products Ltd., Switzerland represented in the EU by Novozymes A/S, Krogshoejvej 36, 2880 Bagsvaerd, Denmark.
[3] FEED dossier reference: FAD-2018-0078.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2017-0046-muramidase.pdf
[5] 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.

3. Assessment

The present opinion deals with the assessment of the safety and efficacy of the enzyme preparation containing muramidase produced by *Trichoderma reesei* DSM 32338 as a zootechnical feed additive (functional group: other zootechnical additives) for turkeys for fattening, turkeys reared for breeding, chickens reared for breeding and other poultry species reared for breeding.

3.1. Characterisation of the additive

The additive, available in solid or liquid form, is a muramidase enzyme preparation (Enzyme Commission Number 3.2.1.17, lysozyme or *N*-acetylmuramidase) which is produced by a genetically modified strain of *T. reesei* deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM) with the accession number DSM 32338. The solid and liquid forms ensure a guaranteed minimum activity of 60,000 LSU(F)[6] per gram. Information relating to the characterisation of the additive and to the genetically modified microorganism has been recently assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2018a).

The applicant provided new data regarding the shelf-life of the additive up to 18 months.[7,8] The shelf-life was studied in samples of at least three batches of each formulation stored at temperatures ranging from 10 to 40°C. The additive in solid formulation showed a mean recovery of the initial activity of 100%, 93% and 64% after 18 months at 10°C, 25°C and 35°C, respectively. The additive in liquid formulation showed 86%, 66% and 42% mean recovery of the initial activity after 18 months at 10, 25 and 35°C, respectively.

3.1.1. Conditions of use

The additive is intended to be added to feed for turkeys for fattening, turkeys reared for breeding, chickens reared for breeding and other poultry species reared for breeding to provide between 25,000 and 45,000 LSU(F) per kg feed. The solid form should be incorporated directly to feed or via premixture. The liquid form is designed to be sprayed directly to the compound feed, in case of pelleting the liquid should be incorporated post-pelleting.

3.2. Safety

The safety aspects regarding the use of this additive in feed including the safety of the genetic modification of the production strain, the safety for the consumers, for the user and for the environment have been previously assessed (EFSA FEEDAP Panel, 2018a). The Panel concluded that no safety concerns would arise from the genetically modified production strain and that the use of the product as a feed additive raises no concerns for consumer safety or for the environment. Regarding the safety for the user, the Panel could not conclude on the potential of the additive for skin/eye irritancy and skin sensitisation. The additive should be considered a potential respiratory sensitiser. The FEEDAP Panel is not aware of any new information that would lead it to reconsider the conclusions drawn previously. Moreover, the FEEDAP Panel considers that the new use requested by the applicant would not introduce hazards/risks not considered in previous assessments for the above aspects.

Since the application includes new species/categories, the safety for these new target species should be assessed.

3.2.1. Safety for the target species

Safety for chickens for fattening and minor poultry species was established in a previous assessment (EFSA FEEDAP Panel, 2018a). In a tolerance study submitted performed in chickens for fattening, the results showed no negative effects on the performance, blood parameters and gross pathology examination of the birds when fed 10× the maximum recommended dose of 45,000 LSU (F)/kg feed. This conclusion is extended to chickens reared for laying/breeding.

[6] 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.
[7] Technical dossier/Section II/Annex II 16.
[8] Technical dossier/Section II/Annex II 17.

Considering that the margin of safety observed in the tolerance study in chickens for fattening is 10, and that the maximum recommended doses in chickens and the target species under application is the same, the Panel extrapolates the conclusions reached in chickens to turkeys for fattening and turkeys reared for breeding and to all other minor poultry species for fattening and reared for laying/breeding.

The applicant calculated the maximum safe concentration for turkeys for fattening in feed based on the no-observed-adverse-effect-level (NOAEL; 384,616 LSU(F)/kg body weight) obtained in the 90-day study in rats according to the guidance on the safety for the target species (EFSA FEEDAP Panel, 2017). The result of the calculation was 57,366 LSU(F)/kg feed and this would support the above conclusion regarding the safety of the additive for turkeys for fattening or reared for laying/breeding.

3.2.1.1. Conclusions on the safety for the target species

The FEEDAP Panel concludes that the additive is safe for turkeys for fattening, turkeys reared for breeding, chickens reared for laying/breeding and other poultry species reared for breeding up to the maximum recommended dose of 45,000 LSU(F)/kg feed.

3.3. Efficacy

The efficacy of muramidase was previously established in chickens for fattening and minor poultry species (EFSA FEEDAP Panel, 2018a) based on efficacy studies in chickens for fattening that showed improvements of the feed to gain ratio at the minimum level of 25,000 LSU(F)/kg feed.

The conclusions from the efficacy studies in chickens for fattening can be extended to chickens reared for laying/breeding and can be extrapolated to turkeys for fattening or reared for breeding and other minor poultry species reared for laying/breeding at the same use level. Therefore, the FEEDAP Panel concludes that the additive has the potential to be efficacious as a zootechnical additive in turkeys for fattening, turkeys reared for breeding, chickens reared for laying/breeding and other poultry species reared for breeding when added to feed at 25,000 LSU(F)/kg feed.

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[9] and Good Manufacturing Practice.

4. Conclusions

Muramidase produced by *Trichoderma reesei* DSM 32338 is considered safe for turkeys for fattening, turkeys reared for breeding, chickens reared for laying/breeding and other poultry species reared for breeding up to the maximum recommended dose of 45,000 LSU(F) per kg feed.

The additive is considered safe for the consumer and the environment. No conclusions can be reached on the potential of the additive for skin/eye irritancy and skin sensitisation. The additive should be considered a potential respiratory sensitiser.

The additive has the potential to be efficacious as a zootechnical additive in turkeys for fattening, turkeys reared for breeding, chickens reared for laying/breeding and other poultry species reared for breeding when added to feed at 25,000 LSU(F)/kg feed.

Documentation provided to EFSA/Chronology

Date	Event
29/10/2018	Dossier received by EFSA. Muramidase produced by Trichoderma reesei DSM 32338. Submitted by DSM Nutritional Products (represented in the EU by Novozymes A/S)
23/11/2018	Reception mandate from the European Commission
16/1/2019	Application validated by EFSA – Start of the scientific assessment
5/3/2019	Comments received from Member States
2/4/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

[9] 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.

References

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 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 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), 2018b. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel(EFSA Panel on additives and products or substances used in animal feed), 2017. 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

Abbreviations

CFU	colony forming unit
DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
NOAEL	no-observed-adverse-effect-level