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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)

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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 (Balancius™) as a feed additive for weaned piglets. The additive has been previously assessed by the FEEDAP Panel in the context of other applications, and in the current assessment the applicant requests for an extension of use. Based on the data available in a sub-chronic oral toxicity study, the Panel concluded that the additive is safe for weaned piglets at the maximum recommended level of 65,000 LSU(F)/kg feed. The additive is safe for the consumers and the environment but should be considered a potential respiratory sensitiser. The Panel could not conclude on the potential of the additive for skin/eye irritancy and skin sensitisation. The additive has the potential to be efficacious as a zootechnical additive for weaned piglets at the dose of 50,000 LSU(F)/kg feed.

Keywords: zootechnical additives, safety, efficacy, muramidase, Balancius™, weaned piglets

Requestor: European Commission

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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 muramidase produced by *Trichoderma reesei* DSM 32338 (Balancius™) when used as a feed additive for weaned piglets (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 20 April 2020.

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

1.2. Additional information

The product under assessment presents muramidase activity and is produced by fermentation with a genetically modified strain of *Trichoderma reesei* (DSM 32338).

The FEEDAP Panel adopted two opinions on the product, one on the safety and efficacy for chickens for fattening and minor poultry species (EFSA FEEDAP Panel, 2018a) and another one on the safety and efficacy for turkeys for fattening, turkeys reared for breeding, chickens reared for breeding and other poultry species reared for breeding (EFSA FEEDAP Panel, 2019a).

This additive is authorised as a zootechnical additive (functional group: other zootechnical additives (improvement of the feed to gain ratio)) for chickens and minor poultry species for fattening[3] and turkeys for fattening, chickens, turkeys and other poultry species reared for breeding.[4]

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[5] in support of the authorisation request for the use of muramidase produced by *Trichoderma reesei* DSM 32338 (Balancius™) 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.

The European Union Reference Laboratory (EURL) 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 for the current application.[6]

[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, Wurmisweg 576, CH-4303, Kaiseraugst, Switzerland represented in the EU by Novozymes A/S Denmark, Krogshoejvej 36, DK-2880, Bagsvaerd, Denmark.

[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 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] FEED dossier reference: FAD-2020-0012.

[6] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2017-0046-muramidase.pdf

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 (Balancius™) is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018c), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019b).

3. Assessment

The present opinion deals with the assessment of the safety and efficacy of the muramidase produced by *Trichoderma reesei* DSM 32338, referred here below as Balancius™, as a zootechnical feed additive (functional group: other zootechnical additives) for weaned piglets.

3.1. Characterisation of the additive

Balancius™ contains muramidase (EC Number 3.2.1.17, lysozyme or N-acetylmuramidase) 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 information relating to the characterisation of the additive, as well as the production strain, has been assessed by the FEEDAP Panel in a previous opinion (EFSA FEEDAP Panel, 2018a). The additive is available in solid and liquid forms and ensure a guaranteed minimum activity of 60,000 LSU(F)[8] per gram of product.

In the context of the current application, the applicant submitted new data regarding the shelf-life of the additive, and on the presence of DNA of the production strain in the additive.

The shelf-life was studied in samples of at least three batches of each formulation stored at 10, 25 and 35°C for up to 24 months.[9] Sub-samples of the same batches were also stored at −18°C for the same period of time and used as reference to calculate the activity recovered. The solid formulation showed a mean recovery of the initial activity of 94, 86 and 54% after 24 months storage at 10, 25 and 35°C, respectively, ensuring compliance to the specifications only at temperature up to 25°C. The additive in liquid formulation showed 86, 63 and 36% mean recovery of the initial activity after 24 months storage at 10, 25 and 35°C, respectively, ensuring compliance with the specifications only at 10°C.

[redacted][10]

[redacted][11]

No DNA from the production strain was detected in the analysed samples

[redacted]

[7] 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.

[8] 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.

[9] Technical dossier/Section II/Annex II.36 and II.37.

[10] Technical dossier/Supplementary information October 2020/Annex 2.

[11] Technical dossier/Supplementary information January 2021/Annexes 1 and 2.

[redacted]

3.1.1. Conditions of use

The additive is to be used in feed for weaned piglets at a minimum recommended enzyme activity of 50,000 LSU(F)/kg feed and a recommended level ranging between 50,000 and 65,000 LSU(F)/kg feed. The solid form is intended to 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 of the genetic modification of the production strain, the safety for the consumers, for the users and for the environment of the use of this feed additive 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 and 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 but concluded that it should be considered a respiratory sensitiser.

The FEEDAP Panel is not aware of any new information that would lead it to reconsider the conclusions drawn previously 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. However, the safety for the new target species/categories sought in the current application needs to be addressed.

3.2.1. 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 for the weaned piglets. In that study a no observed adverse effects level (NOAEL) of 384,616 LSU(F)/kg body weight and day in rats was identified. 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 weaned piglets is calculated to be 76,923 LSU(F)/kg feed. This value would allow to conclude that the additive is safe for piglets at the maximum recommended level of 65,000 LSU(F)/kg.

3.3. Efficacy

Three efficacy trials, carried out at two different locations, were submitted to support the efficacy in weaned piglets. The design of these trials is presented in Table 1 and the results in Table 2. In all the studies, weaned piglets were penned in groups (same number of females and castrated males per pen) and considered at least two experimental groups: one receiving the basal diets (starter and grower) not supplemented and another one receiving the basal diets supplemented with the additive in the solid form in order to provide 50,000 LSU/(F)/kg feed. In the second trial there was a third experimental group that considered a concentrated form of the product (c-CT), which was added to the feed to provide 50,000 LSU/(F)/kg feed. The enzyme activities were confirmed by their analysis in the feed (Table 1). The diets were offered to the animals in mash form and on *ad libitum basis* for 42 days. Health status was monitored throughout the experimental period. Feed intake and body weight of the animals were measured and feed to gain ratio and average daily gain calculated. In all studies an analysis of variance (ANOVA) was performed on the data. The pen was the experimental unit. For trial 2 group means were compared using Tukey test. Significance was established at $p < 0.05$.

Table 1: Trial design and enzyme levels in the efficacy trials in weaned piglets

Trial	Total no of animals (animals × replicate) replicates × treatment	Breed sex (duration)	Composition feed (form)	Groups (LSU(F)/kg feed)	
				Intended	Analysed
1[(a)]	1,200 10 60	Danbred × Duroc males and females (42 days)	Wheat, barley, soybean meal (milk powder in the starter) (mash)	0 50,000	6,289 63,714
2[(b)]	120 2 20	Danbred × Piétrain males and females (42 days)	Wheat, barley, soybean meal (milk powder in the starter) (mash)	0 50,000 GT 50,000 c-CT	4,083 72,153 64,995
3[(c)]	1,280 10 64	Danbred × Duroc males and females (42 days)	Wheat, barley, soybean meal (milk powder in the starter) (mash)	0 50,000	8,403 58,948

(a): Technical dossier/Section IV/Annexex IV.6 to IV.10 and supplementary information October 2020/Annex 3.
(b): Technical dossier/Section IV/Annexex IV.11 to IV.15 and supplementary information October 2020/Annex 4.
(c): Technical dossier/Section IV/Annexex IV.16 to IV.19 and supplementary information October 2020/Annex 4.

The piglets that received the additive at the minimum recommended dose had a significant higher average daily weight gain in trial 1, and a significant better feed to gain ratio in all three trials. Therefore, the FEEDAP Panel concludes that Balancius™ is efficacious in weaning piglets at the minimum proposed use level of 50,000 LSU(F)/kg feed.

Table 2: Effects of Balancius™ on the performance of weaned piglets for the 42 days under study

Trial	Groups (LSU (F)/kg feed)	Total feed intake (kg)	Initial body weight (kg)	Final body weight (kg)	Average daily weight gain (g)	Feed to gain ratio	Mortality and culling (%)
1	0	35.8	8.7	30.6	521[a]	1.64[a]	0.5
	50,000	36.0	8.7	31.4	541[b]	1.60[b]	0.8
2	0	34.7	7.5	29.1	514	1.61[a]	0
	50,000 GT	34.3	7.5	29.4	520	1.57[b]	0
	50,000 c-CT	34.1	7.5	29.7	526	1.54[b]	0
3	0	29.3	8.2	27.3	456	1.54[a]	0.6
	50,000	29.7	8.2	27.9	470	1.51[b]	0.8

[a,b]: Mean values within a trial and within a column with a different superscript are significantly different $p < 0.05$.

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[12] and Good Manufacturing Practice.

4. Conclusions

Balancius™ is safe for weaned piglets up to the maximum recommended level of 65,000 LSU(F)/kg feed.

Balancius™ used as feed additive for weaned piglets is safe for the consumers and the environment. The additive should be considered a respiratory sensitiser, but the Panel cannot conclude on the potential of the additive for skin/eye irritancy and skin sensitisation.

The additive has the potential to be efficacious as a zootechnical additive in weaned piglets at the minimum recommended level of 50,000 LSU(F)/kg feed.

[12] 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.

5. Documentation as provided to EFSA/Chronology

Date	Event
10/02/2020	Dossier received by EFSA. Balancius extension of EU authorization. DSM Nutritional products Ltd. Switzerland represented in EU by DSM Nutritional Products Sp.z o.o.
09/03/2020	Reception mandate from the European Commission
20/04/2020	Application validated by EFSA – Start of the scientific assessment
14/07/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterization, safety for the user and efficacy*
20/07/2020	Comments received from Member States
09/10/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
27/01/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ANOVA	analysis of variance
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
NOAEL	no observed adverse effects level