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Safety and efficacy of Beltherm MP/ML (endo-1,4-beta-xylanase) as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, turkeys for breeding purposes and minor poultry species

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Abstract

Beltherm MP/ML contains endo-1,4-beta-xylanase and is intended to be used as a zootechnical additive for poultry species. The safety and efficacy of the additive have been evaluated by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) in 2017. In that assessment, the additive and the production strain, a genetically modified strain of *Bacillus subtilis*, were characterised in full. No viable cells of the production strain were detected in the final additive, but recombinant DNA from the production strain was detected. Considering that the production strain does not carry genetic sequences of concern, the presence of DNA was considered *per se* of no safety concern by the Panel. The safety of the additive for the target species, consumers and environment was established, but the Panel concluded that the additive should be considered as a respiratory sensitiser. Regarding the efficacy, it was concluded that the additive has a potential to be efficacious in chickens for fattening and extended/extrapolated the conclusion to chickens reared for laying and minor poultry species for fattening. However, the Panel could not conclude on the efficacy of the additive in turkeys for fattening, reared for breeding or for breeding purposes. The Panel concluded, based on the new guidance on the efficacy for feed additives, that the conclusion on the efficacy in chickens for fattening can be extrapolated to turkeys for fattening/reared for breeding purposes. New data in turkeys for fattening would support the extrapolation. No data has been provided to support the efficacy in turkeys for breeding purposes and therefore no conclusion could be drawn. Finally, the applicant has proposed, in the current submission, a modification of the manufacturing process to remove the presence of DNA from the production strain in the final product, the removal was confirmed by analyses.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003 establishes rules governing the Community authorisation of additives for animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Puratos, is seeking a Community authorisation of endo-1,4-beta-xylanase (IUB: EC 3.2.1.8) as a feed additive to be used as a digestibility enhancer for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, turkeys for breeding purposes and minor poultry species (Table 1).

Table 1: Description of the substances

Category of additive	Zotechnical additive
Functional group of additive	Digestibility enhancers
Description	Endo-1,4-beta-xylanase (IUB: EC 3.2.1.8)
Target animal category	Chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, turkeys for breeding purposes and minor poultry species
Applicant	Puratos
Type of request	New opinion

On July 2017, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ('Authority'), in its opinion on the safety and efficacy of the product,¹ confirmed the presence of rDNA in the final product which makes the additive falling under the Regulation (EC) No 1829/2003². The Panel could also not conclude on the efficacy of endo-1,4-beta-xylanase (IUB: EC 3.2.1.8) in turkeys for fattening or reared for breeding and in turkeys for breeding purposes, under the conditions of use as proposed by the applicant.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment to allow a revision of Authority's opinion. The new data have been received on 16 July 2018.

In view of the above, the Commission asks the Authority to deliver a new opinion on endo-1,4-beta-xylanase (IUB: EC 3.2.1.8) as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding turkeys for breeding purposes and minor poultry species based on the additional data submitted by the applicant.

1.2. Additional information

The additive under assessment is Beltherm MP/ML and contains an endo-1,4-beta-xylanase which is produced by a genetically modified strain of *Bacillus subtilis* (BCCM LMG S-27588). The FEEDAP Panel adopted an opinion in 2017 regarding the safety and efficacy of Beltherm MP/ML as a feed additive for poultry species (EFSA FEEDAP Panel, 2017).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information³ to a previous application on the same product.⁴

¹ <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.4941>

² Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed.

³ FEED dossier reference: FAD-2018-0060.

⁴ FEED dossier reference: FAD-2016-0010.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the efficacy of Beltherm MP/ML is in line with the principles laid down in Regulation (EC) No 429/2008⁵, the Guidance on zotechnical additives (EFSA FEEDAP Panel, 2012a), the technical guidance on tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011) and the Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a,b).

3. Assessment

The additive Beltherm MP/ML contains endo-1,4-beta-xylanase (Enzyme Commission number: 3.2.1.8 xylanase) and is available in two forms, solid and liquid, which ensure a minimum activity of 500 ADXU⁶/g product. The xylanase present in the additive is produced by a genetically modified strain of *Bacillus subtilis* which is deposited at the Belgian Co-ordinated Collections of Microorganisms, University of Ghent, with the deposition number BCCM- LMG S-27588. It is intended to be used as a zotechnical additive (digestibility enhancers) for chickens for fattening or reared for laying, turkeys for fattening or reared for breeding, turkeys for breeding purposes and minor poultry species.

The EFSA FEEDAP Panel assessed the safety and the efficacy of the additive for poultry species (EFSA FEEDAP Panel, 2017). In that initial assessment, the additive and the production strain were characterised and described in full. The production strain and the genetic modification raised no safety concerns. No viable cells of the production strain were detected in the final additive, but recombinant DNA was detected. However, the presence of the DNA from the production strain was considered, *per se*, to raise no safety concerns by the Panel. The safety of the additive for the target species, consumers and the environment were established. Considering the safety for the user the Panel concluded that the additive, in either form, is not toxic by inhalation or irritant for skin or eye but it is considered a potential respiratory sensitiser. The Panel could not conclude on the dermal sensitisation potential of the additive. The Panel also concluded that the additive has a potential to be efficacious in chickens for fattening but could not conclude on the efficacy of the additive in turkeys for fattening, reared for breeding or for breeding purposes.

The applicant has provided new data to support the efficacy of the additive in turkeys and has proposed a modification on the manufacturing process of the additive which aims at removing the DNA of the production strain in the final product.

3.1. Characterisation of additive

The production strain, the manufacturing process and the resulting product were evaluated and described in 2017 by the FEEDAP Panel (EFSA FEEDAP Panel, 2017). The production strain *B. subtilis* BCCM LMG S-27588 was unequivocally identified; the absence of toxigenicity was shown on Vero cells and the strain was found to be susceptible to all the antibiotics listed for *B. subtilis* in the Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012a,b). The inserted traits during the genetic modification did not raise any safety concern. Moreover, no antibiotic resistance genes used during the genetic modification remain in the production strain. Analyses revealed that the production strain was not detected in the additive but DNA from the production strain remained in the enzyme concentrate. Considering that the production strain does not carry gene sequences that would raise a safety concern, the Panel concluded that the presence of recombinant DNA from the production strain in the additive was, *per se*, of no safety concern. However, the presence of DNA from a genetically modified production strain would require an authorisation under Regulation (EC) No 1829/2003. The applicant has now proposed a modification of the manufacturing process of the additive to ensure the removal of DNA from the production strain during the downstream process.

The original manufacturing process assessed in 2017 consisted of the following steps after the fermentation: microfiltration [REDACTED], ultrafiltration and sterile filtration. [REDACTED]

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. 1–65.

⁶ One ADXU is defined as the amount of enzyme which liberates one micromole of reducing sugars (xylose equivalents) from beechwood xylan per minute at pH 6.0 and at 70°C.

statistically significant. The final body weight was 16.75 and 17.00 kg, and the feed to gain ratio was 2.32 and 2.27 for control and 100 ADXU/kg groups, respectively. The differences between the control and 100 ADXU/kg feed group were statistically significant for the body weight and the feed to gain ratio. The results from this trial would further support the efficacy of the additive in turkeys for fattening when administered at 100 ADXU/kg feed.

3.2.2. Efficacy in turkeys for breeding purposes

No data has been submitted to support the efficacy of the additive in turkeys for breeding purposes, therefore the Panel cannot conclude on the efficacy.

4. Conclusions

The modification of the manufacturing process ensures that no recombinant DNA is present in the additive.

Beltherm MP/ML has a potential to be efficacious as a zootechnical additive in turkeys for fattening or reared for breeding at 100 ADXU/kg feed. The Panel cannot conclude on the efficacy of the additive in turkeys for breeding purposes.

Documentation provided to EFSA

- 1) Beltherm MP/ML for poultry species. August 2018. Submitted by Puratos.
- 2) Beltherm MP/ML for poultry species. Supplementary information. January 2019. Submitted by Puratos.

Chronology

Date	Event
9/8/2018	Dossier received by EFSA
9/8/2018	Reception mandate from the European Commission
19/12/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation and efficacy</i>
16/1/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
23/1/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740, 10 pp. <https://doi.org/10.2903/j.efsa.2012.2740>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2017. Scientific Opinion on the safety and efficacy of Beltherm MP/ML (endo-1,4-beta-xylanase) as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, turkeys for breeding purposes and minor poultry species. EFSA Journal 2017;15(7):4941, 17 pp. <https://doi.org/10.2903/j.efsa.2017.4941>
- 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. 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>

Abbreviations

BCCM	Belgian Co-ordinated Collections of Microorganisms
EC	Enzyme Commission
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed