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 sensitisier. 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 | Zootechnical 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, confirmed the presence of rDNA in the final product which makes the additive falling under the Regulation (EC) No 1829/2003. 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 information to a previous application on the same product.

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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 429/2008\(^5\), the Guidance on zootechnical 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 ADXU\(^6\)/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 zootechnical 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 sensitisier. 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, ultrafiltration and sterile filtration.

\(^5\) 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.
Therefore, the modification of the manufacturing process would allow the removal of the DNA of the production strain from the additive.

3.2. Efficacy

The additive is to be used in the target species at 100 ADXU/kg feed. In the opinion from 2017, the Panel concluded that the additive has a potential to be efficacious in chickens for fattening at the recommended level and extended/extrapolated the conclusion to chickens reared for laying and to minor poultry species for fattening.

However, the Panel could not conclude on the efficacy of the additive in turkeys for fattening or reared for breeding due to the limited evidence to support the efficacy. Significant and positive effects of the additive were found in only two trials. In one trial, the turkeys receiving Beltherm at 100 ADXU/kg feed were significantly heavier. The Panel noted, however, that the statistical comparison of the group means was performed with least significant difference test which does not correct for multiple comparisons and since there were three groups such correction would be desired to compare the group means. In another trial, turkeys receiving Beltherm at 200 ADXU/kg (analysed activity similar to 100 ADXU/kg feed) showed a better feed to gain ratio.

The Panel could not conclude either on the efficacy of the additive for turkeys for breeding purposes because no efficacy data was made available.

3.2.1. Efficacy in turkeys for fattening

The guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a,b) establishes that data from efficacy studies in chickens for fattening can be extrapolated to other poultry species, including turkeys for fattening provided that the mode of action can be reasonably presumed to be the same between the species. In the previous opinion (EFSA FEEDAP Panel, 2017), the FEEDAP Panel concluded that the additive has the potential to improve the performance of chickens for fattening at the dose of 100 ADXU/kg feed. Since the mode of action of xylanases is very well known and considered to be the same among poultry species the conclusion drawn in chickens for fattening can be extrapolated to turkeys for fattening or reared for breeding purposes.

The applicant provided in the current submission a new efficacy trial done in turkeys for fattening in which a total of 288 one-day-old male turkeys (BUT 10) were distributed in 16 pens (18 birds per pen). Two treatments were allocated to the pens, representing eight replicates per treatment. Four basal diets (starter, grower and finisher diets) based on wheat, rye, soya bean meal were either not supplemented or supplemented with the xylanase from Beltherm to provide 100 ADXU/kg feed (analysis showed values from 69 to 88 ADXU/kg feed). The birds received the feed on ad libitum basis and for 112 days. Birds were weighed at the start, days 29, 57, 85 and 112 (individual basis), cumulative feed intake was registered at the same days and feed to gain ratio was calculated for the corresponding periods. Due to a higher growth performance than the expected and in order to reduce the density per pen, on day 28, two birds per pen were removed from study and on day 57 another bird per pen was reduced. An analysis of variance was performed on the performance data considering the pen as the statistical unit. The significance level was set at 0.05. Mortality was below 4% with no difference found between the groups. Total feed intake of the birds during the period was 39.7 and 38.9 kg for control and 100 ADXU/kg feed, respectively; the difference between the groups was not
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. Issues: characterisation and efficacy |
| 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), 2018b. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16 (5):5274, 25 pp. https://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