Safety and efficacy of Beltherm MP/ML (endo-1,4-beta-xylananase) 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 is a feed additive that contains endo-1,4-beta-xylanase to be used as a zootchnical additive in poultry species. The production strain of the xylanase is a genetically modified strain of Bacillus subtilis. This species is considered by EFSA to qualify for the Qualified Presumption of Safety (QPS) approach to safety assessment. The strain was unequivocally identified, qualifications were met and the genetic modification raised no concerns. The production strain was not detected in the enzyme concentrate and therefore would not be present in the additive. Although the presence of DNA was shown in the enzyme concentrate, this is not considered a safety concern. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the additive is safe for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, turkeys for breeding purposes and minor poultry species at 100 ADXU per kg feed with a wide margin of safety. The Panel also concluded that no toxicological concerns for the consumer would arise from the use of Beltherm as a feed additive. The additive is considered a potential respiratory sensitiser for users. The active substance of the additive is of no concern for the environment and the presence of DNA in the enzyme concentrate is not considered a safety concern for the environment. The additive has the potential to improve the performance of chickens for fattening, chickens reared for laying and minor growing poultry species at the dose of 100 ADXU/kg feed. The Panel could not conclude on the efficacy of the product in turkeys for fattening or reared for breeding or in turkeys for breeding purposes.

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Keywords: safety, efficacy, zootchnical additives, digestibility enhancers, xylanase, poultry

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Note: This scientific opinion is published following the EC decision on the confidentiality claims submitted by the applicant as per Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The sections amended are identified in the opinion. The original version has been removed from the EFSA Journal.
Summary

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 Beltherm MP/ML 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.

Beltherm is a feed additive with endo-1,4-beta-xylanase as the declared enzymatic activity, that is available in solid (MP) and liquid forms (ML). The production strain of the xylanase is a genetically modified strain of *Bacillus subtilis*. This species is considered by EFSA to qualify for the QPS approach to safety assessment. The strain was unequivocally identified, qualifications were met and the genetic modification raised no concerns. The production strain was not detected in the enzyme concentrate and therefore would not be present in the additive. Although the presence of DNA was shown in the enzyme concentrate, this is not considered a safety concern.

The results of the tolerance studies showed no negative effects on the performance of chickens for fattening and turkeys for fattening when fed at 200 or 150-fold the recommended dose, respectively. Therefore, the Panel concludes that the additive is safe for these species/categories at the dose of 100 ADXU per kg feed. This conclusion can be extended to chickens reared for laying and turkeys reared for breeding. Based on a study on laying hens the Panel concludes that the additive is safe for turkeys for breeding purposes at the dose of 100 ADXU/kg feed. The wide margin of safety shown would allow this conclusion to be extrapolated to minor poultry species.

Toxicological studies are not required if the fermentation products are produced by a genetically modified microorganism for which the recipient strain is considered by EFSA to qualify for the QPS approach to safety assessment and for which the genetic modification raises no concerns. The enzyme is produced by a genetically modified strain of *B. subtilis*; this species is considered to qualify for the QPS approach to safety assessment provided that the qualifications are met. The identity of the strain was unambiguously established, qualifications were met and the genetic modification raised no concerns. Therefore, the Panel concluded that the product is of no concern for the consumer of food obtained from animals fed with it. This conclusion was supported by the results obtained in the toxicological tests conducted with an intermediate of the additive.

The assessment of the safety for the user was done with an intermediate product and not with the final formulations. The intermediate product did not prove to be toxic by inhalation or irritant for skin or eye and the ingredients used to formulate the additive are not likely to contribute to the irritant properties. Owing to the lack of data, the Panel could not conclude on the dermal sensitisation potential of the additive. Because of the proteinaceous nature of the active substance, the additive was considered a potential respiratory sensitiser.

The active substance of the additive is a protein, and as such will be degraded/inactivated during passage through the digestive tract of animals or in the environment. The production strain was not detected in the concentrate and therefore it would not be detected in the additive. Although the presence of DNA was shown in the enzyme concentrate, this is not considered a safety concern. Therefore, the Panel concludes that the additive does not raise concerns regarding the environment.

Efficacy studies in chickens and turkeys for fattening were evaluated. Based on the results of a statistical analysis that pooled the data from four efficacy trials, 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. The conclusion was extended to chickens reared for laying. The mode of action of xylanases is well known and considered to be similar among poultry species, therefore the conclusion was extrapolated to minor growing poultry species. Due to the limited evidence in turkeys for fattening the Panel could not conclude on the efficacy of the product in turkeys for fattening or reared for breeding. The Panel could not conclude on the efficacy in turkeys for breeding purposes due to the lack of data.
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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ 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 Puratos² for authorisation of the product Beltherm MP/ML (endo-1,4-beta-xylanase), when used as a feed additive for chickens for fattening, chickens reared for laying, minor poultry species, turkeys for breeding purposes, turkeys reared for breeding, turkeys for fattening (category: zootechnical additives; functional group: digestibility enhancers).

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). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 25 May 2016.

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 Beltherm MP/ML (endo-1,4-beta-xylanase), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

Beltherm MP/ML is an additive that presents endo-1,4-beta-xylanase activity which has not been authorised in the European Union. The enzyme present in the product is produced by a genetically modified strain of Bacillus subtilis. The species B. subtilis is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to establishing safety for the target species, consumers and the environment (EFSA, 2007; EFSA BIOHAZ Panel, 2017). This approach requires the identity of the strain to be established and the absence of toxigenic potential and susceptibility to a selected range of antibiotics to be demonstrated.

2. Data and Methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier³ in support of the authorisation request for the use of Beltherm as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008⁴ and the applicable EFSA guidance documents.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁵

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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 Puratos, Industrialaan 25, 1702 Groot-Bijgaarden, Belgie.
³ FEED dossier reference: FAD-2016-0010.
⁴ 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.
⁵ The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finirep-fad-2016-0010-beltherm.pdf
2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Beltherm is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c), Guidance on the assessment of the toxigenic potential of Bacillus species used in animal nutrition (EFSA FEEDAP Panel, 2014), Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008b), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA, FEEDAP Panel 2012d), Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011).

3. Assessment

Beltherm is an additive which contains endo-1,4-beta-xylanase (EC 3.2.1.8; xylanase) and is intended to be used as a zootechnical additive (functional group: digestibility enhancers) in chickens and turkeys for fattening, chickens and turkeys reared for laying/breeding, turkeys for breeding purposes and minor poultry species.\(^6\)

3.1. Characterisation

3.1.1. Characterisation of the active substance

The xylanase present in the product is produced by a genetically modified strain of Bacillus subtilis.

3.1.1.1. Information relating to the genetically modified microorganism\(^7\)

The production organism is a genetically modified strain of B. subtilis, deposited at the Belgian Coordinated Collections of Microorganisms, University of Ghent, with deposition number LMG S-27588.\(^8\) The recipient strain was identified as B. subtilis by 16S rRNA gene sequence analysis\(^9\). Cytotoxicity of B. subtilis LMG S-27588 was assessed on Vero cells in accordance to the FEEDAP Panel guidance document (EFSA FEEDAP Panel, 2014) and gave negative results.\(^10\) The sensitivity of the production strain to antimicrobials was tested by microdilution as recommended by EFSA (EFSA FEEDAP Panel, 2012d). The technical dossier contains detailed and sufficient information on the parental/recipient microorganism, the origin and function of the different genetic elements introduced/ modified in the production strain, the genetic modification process and the outcome of the genetic modifications.

3.1.2. Manufacturing process\(^11\)

The enzyme is produced by submerged fermentation followed by a series of concentration and purification steps.

3.1.3. Characterisation of the additive

The additive is available in solid or liquid form and the two forms ensure a guaranteed minimum activity of 500 ADXU\(^12\)/g of product.

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\(^6\) The applicant clarified that laying hens are not within the scope of this application.

\(^7\) This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

\(^8\) Technical Dosier/Supplementary information February 2017/Annex Q01.

\(^9\) Technical Dosier/Section II/Annex II.16.

\(^10\) Technical Dosier/Section II/Annex II.19.

\(^11\) This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

\(^12\) One ADXU is defined as the amount of enzyme which literates one micromole of reducing sugars (xylose equivalents) from beechwood xylan per minute at pH 6.0 and at 70 °C.
The batch to batch variation of the solid formulation, Beltherm MP, was studied in 8 batches and the mean value was 519 ADXU/g, ranging from 468 to 548 ADXU/g (coefficient of variation (CV) of 5.4 %). Two of the batches analysed were below the specifications. The solid formulation contains the (spray-dried) enzyme concentrate and food grade wheat flour (~ 90 % weight/weight). The particle size was studied in four batches by laser diffraction. The mean particle size was 129 μm, with up to 23% of particles below 100 μm, up to 5.2% of particles below 50 μm, and less than 0.03% of particles below 5 μm. The dusting potential was measured (Stauber-Heubach) in three samples and the values were 42, 0 and 7 mg/m².

The batch to batch variation of the liquid formulation was studied in 11 batches and the mean value was 574 ADXU/g, ranging from 494 to 703 ADXU/g (coefficient of variation (CV) of 14.8%). Two of the batches evaluated were below the specifications. This formulation contains, according to the applicant, the enzyme concentrate, glycerol (maximum of 30 % w/w), acetate buffer (min of 70 % w/w) and potassium sorbate (0.1 %). The viscosity of this formulation was measured in five batches and the mean was 5.41 cP (measured at 25 °C), the pH varied from 4.5 to 4.7 and the bulk density was similar to 1,008 kg/m³.

Five batches of the liquid concentrate and three batches of the solid and liquid formulations were analysed for chemical and microbiological contamination. The analysis of chemical contamination included arsenic (<0.1 mg/kg), cadmium (<0.02 mg/kg), mercury (<0.01 mg/kg), lead (0.1 mg/kg), aflatoxin B1, B2, G1 and G2 (< 1 μg/kg), T2-toxin (<20 μg/kg), ochratoxin A (<0.5 μg/kg), zearalenone (< 10 μg/kg) and pesticides. Microbiological analysis included total aerobic counts (≤100 CFU/g for the liquid concentrate, 9x10³ to 28x10³ CFU/g for the liquid formulation and <700 CFU/g for the solid), coliform bacteria (<10 CFU/g), *Staphylococcus aureus* (absence per g), clostridia sulphite reducers (<10 CFU/g), *Salmonella spp.* (absence in 25 g) and *Escherichia coli* (absence in one g). Antimicrobial activity was not detected when analysed following the method specified by the Joint FAO/WHO Expert Committee on Food Additives (FAO JECFA, 2006).

The production strain could not be detected in a test volume of 1 mL of three batches of the liquid concentrate before formulation, each sample tested in triplicate. Culturing included a resuscitation step in non-selective liquid medium for 24 hours at 35 °C, followed by plating of 100 μl on solid medium that allowed the specific detection of xylanase-producing microorganisms and incubation for 16h at 35°C.

A 1,074-bp fragment corresponding to the recombinant xylanase gene and a sequence encoding the signal peptide of the production strain *B. subtilis* LMG S-27588 was detected by PCR in three batches of the concentrate before formulation (liquid, enzyme activity ranging from 696 to 1,211 ADXU/mL). Therefore, the additive contains recombinant DNA from the production strain.

3.1.4. **Stability and homogeneity**

The shelf-life of the solid and liquid formulations was studied in samples kept in the closed original containers at 25 or 40 °C up to twelve months. The shelf-life of the additive is claimed to be 12 months for the solid formulation and 9 months for the liquid when stored at 25 °C. Three batches were tested and showed that the enzyme recovery after 12 months was > 90% for the solid formulation, regardless of the temperature. For the liquid, the recoveries after 9 months were ~ 90% when stored at 25 °C and recoveries ranged from 40 to 75% when stored at 40 °C.

Three batches of the solid formulation were mixed with a vitamin and mineral premixture containing choline chloride for chickens for fattening at a rate of 10,000 ADXU/g premixture. Samples were kept at room temperature (temperature range 10 to 25 °C) in closed containers. Recoveries after six months were >97%. The same batches were mixed in mash feed for chickens for fattening at an

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13 Technical dossier/Section II/Annex II.03.
14 Technical dossier/Section II/Annex II.03.
15 Technical dossier/Section II/Annex II.03.
16 Technical dossier/Section II/Annex II.03.
17 Technical dossier/Section II/Annex II.05.
18 Technical dossier/Section II/Annex II.20.
19 Technical dossier/Section II/Annex II.28 and Supplementary information February 2017.
20 Technical dossier/Section II/Annex II.29 and supplementary information February 2017.
intended dose of 100 ADXU/kg feed. This feed was subject to pelleting at different temperatures (80, 85, 90 and 95 °C) and the recoveries were measured. Samples of mash and pelleted feed (treated at 90 °C) were kept for three months at room temperature (temperature range 10 to 25 °C) in closed containers in order to study the stability. The average enzyme recoveries after pelleting (mash vs pelleted) were 83, 88, 81 and 80% for 80, 85, 90 and 95 °C, respectively. Recoveries in stored feed after 3 months showed no losses of enzyme activity irrespective of the form of the feed (mash or pelleted).

Three batches of the liquid formulation were sprayed on a mash or a pelleted feed for chickens for fattening at an intended dose of 100 ADXU/kg. Samples of the feed were kept for three months at room temperature (temperature range 10 to 25°C) in closed containers. Recoveries after three months essentially showed no losses of enzyme activity.

The capacity of the two forms of the additive to homogeneously distribute in feed was studied for one batch of the solid formulation in pelleted and mash feed and for three batches of the liquid in pelleted feed. The enzyme supplementation was 100 ADXU/kg feed and ten subsamples of the feeds were analysed. The coefficient of variation was of 3.8 % for the solid formulation in mash feed and of 3.0 % for the pelleted feed and ranged from 4.0 to 4.7 % for the three batches of the liquid formulation in the pelleted feed.

3.1.5. Conditions of use

The additive is to be added to the feed for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, turkeys for breeding purposes and minor poultry species to provide 100 ADXU per kg feed.

3.2. Safety

3.2.1. Safety aspects of the production strain

B. subtilis qualifies for the QPS approach to safety assessment provided that it is unequivocally identified and that the qualifications are met. The production strain B. subtilis LMG S-27588 was unequivocally identified; absence of toxigenicity was shown on Vero cells and the strain is susceptible to all relevant antibiotics. The inserted traits do not raise safety concern. No antibiotic resistance genes used during the genetic modification remain in the production strain. The gene present in B. subtilis is not considered to be a gene of concern (Section 3.1.1.1.).

Analyses revealed that the production strain was not present in the final products. Although the presence of DNA was shown in the enzyme concentrate, this is not considered a safety concern. Therefore, the additive does not raise safety concern with respect to the production strain and its genetic modification.

3.2.2. Safety for the target species

3.2.2.1. Safety for chickens for fattening

A total of 2,208 one-day-old male chickens for fattening (Ross PM3) were distributed in 48 pens in groups of 46 animals and allocated to four dietary treatments (representing 12 replicates per treatment). Two basal diets (starter and grower) based on wheat, soya bean meal, and maize were either not supplemented (control) or supplemented with the xylanase from Beltherm to provide 100 (1× recommended dose), 200 (2×) or 20,000 (200×) ADXU per kg feed (confirmed by analysis). Diets were offered in pelleted form for 36 days. Mortality was checked every day and dead animals were necropsied. Animals were weighed at the start (pen basis) and on days 22 and 36 (individual basis), feed intake was registered per pen and feed to gain ratio was calculated per pen. An analysis of variance (ANOVA) was done with the performance data considering the pen as the statistical unit.

21 Technical dossier/II.30 and supplementary information February 2017.
22 Technical dossier/II.30 and supplementary information February 2017.
23 Technical dossier/II.31 and Supplementary information February 2017.
24 This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003
25 Technical dossier/III.02 and III.05.1 and Supplementary information February 2017/Annex Q17.
Group means were compared with Dunnett test. Mortality was analysed using the Kruskall-Wallis method. Significance level was set at 0.05.

Mortality and culling rate was 5% and no differences were found between treatments. Mean daily feed intake was 103 g, final body weight was 2.31 kg and feed to gain ratio was 1.58. Significant differences between treatments were observed (in favour of the xylanase, for further details see efficacy trial 2). Feeding the birds with the xylanase up to 200-fold the recommended dose did not have any negative effects on the performance parameters.

3.2.2.2. Safety for turkeys for fattening

A total of 720 one-day-old male turkeys for fattening (BUT 9) were distributed in 24 pens in groups of 30 animals and allocated to four dietary treatments (representing 6 replicates per treatment). Two basal diets (starter and grower) based on wheat and soya bean meal were either not supplemented (control) or supplemented with the xylanase from Beltherm to provide 100 (1×), 200 (2×) or 20,000 (200×) ADXU per kg feed. The analysed enzyme activities were 97, 178 and 15,400 for 1×, 2× and 200×. Diets were offered for 56 days, as mash in the starter phase and as pellets in the grower phase. Health status of the birds and mortality were monitored twice daily. Animals that died during the first week were replaced by birds that received the control diet. Animals were weighed at the start, day 27 and day 56 (pen basis), feed intake was registered for periods 0-27 and 27-56 and feed to gain ratio was calculated. An analysis of variance was performed on the performance data considering the pen as the statistical unit and the group means were compared with Dunnett test. Significant level was set at 0.05.

The mortality and culling was 10.5, 5.0, 7.7, 9.4% for control, 100, 200 and 20,000 group, respectively. Most of the mortality happened in the first week of study (small animals) and no differences were found between treatments. Mean daily feed intake was 142, 143, 146 and 133 g for control, 1×, 2× and 200×, respectively. The corresponding figures for final body weight were 4.90, 4.93, 5.03 and 4.77 kg and for feed to gain ratio they were 1.65, 1.64, 1.64 and 1.58. No significant differences were observed on the body weight of the groups receiving the xylanase compared to the control. A lower feed intake and better feed to gain ratio were observed in the 200× group (analysed dose 150-fold) compared to the control. Feeding the birds with the xylanase up to 150-fold the recommended dose did not have any negative effects on the performance parameters.

3.2.2.3. Safety for turkeys for breeding

No specific data were provided in turkeys for breeding purposes; however, the applicant provided a tolerance study in laying hens that could provide some supporting evidence on the safety of the additive for turkeys for breeding.

A total of 576 22 week-old hens (Hy-Line Brown) were distributed in 72 enriched cages of 8 hens each and were allocated to six dietary treatments (12 replicates (cages) per treatment). A basal diet based on wheat, rye and soya bean meal was either not supplemented (control) or supplemented with the xylanase from Beltherm to provide 100, 125, 200 or 20,000 ADXU per kg feed. The enzyme activities were confirmed by analysis. The sixth experimental group considered another xylanase. The feed was offered in mash form and ad libitum for 24 weeks. Mortality and general health were monitored throughout the study. Body weight per replicate (cage) was recorded at the beginning and at the end of the trial. Feed consumption was recorded every four weeks. Egg production per cage was recorded and weighed every second day. Feed to egg mass ratio was calculated. The number of broken, shell-less and dirty eggs was recorded. An ANOVA was performed on the data obtained, cage basis, and differences were considered significant at a level of at least P < 0.05. Group means were compared with Duncan test.

No mortality was reported. Mean daily feed intake of the hens was 122 g, laying rate was 95 %, daily egg mass was 62 g and feed to egg mass conversion ratio was 1.99. The group receiving 20,000 ADXU/kg feed showed significant and better feed to egg mass conversion ratio than the control due to a lower feed intake. Therefore, feeding the hens with the xylanase up to 20,000 ADXU/kg feed did not have any negative effects on the performance parameters.

26 Technical dossier/Section III/Annexes III.03 and III.05.2. and Supplementary information February 2017/Annex Q18.
27 Technical dossier/Section III/Annex III.04 and III.05.3.
The Panel considers that the results from this study in the laying hens can support the safety for turkeys for breeding purposes at the dose of 100 ADXU per kg feed.

3.2.2.4. Conclusions on safety for the target species

The results of the tolerance studies showed no negative effects on the performance of chickens for fattening and turkeys for fattening when fed at 200 or 150-fold the recommended dose, respectively. Therefore, the Panel concludes that the additive is safe for these species/categories at the dose of 100 ADXU per kg feed. This conclusion can be extended to chickens reared for laying and turkeys reared for breeding.

Based on a study on laying hens the Panel concludes that the additive is safe for turkeys for breeding purposes at the dose of 100 ADXU/kg feed.

The wide margin of safety shown would allow this conclusion to be extrapolated to minor poultry species.

3.2.3. Safety for the consumer

Toxicological studies are not required if the fermentation products are produced by a genetically modified microorganism for which the recipient strain is considered by EFSA to qualify for the QPS approach to safety assessment and for which the genetic modification raises no concerns.

The enzyme is produced by a genetically modified strain of *B. subtilis*; this species is considered to qualify for the QPS approach to safety assessment provided that the qualifications are met. The identity of the strain was unambiguously established, qualifications were met and the genetic modification applied raised no concerns, therefore, the FEEDAP Panel considers that the additive is safe for the consumer of food derived from animals that received the additive.

This conclusion is supported by the results obtained in the toxicological tests conducted with the fermentation product before formulation: three genotoxicity studies and one sub-chronic oral toxicity study. A bacterial reverse mutation assay performed with *Salmonella* Typhimurium strains TA98, TA100, TA102, TA1535 and TA1537 in accordance with OECD Guideline 471 showed no evidence of mutagenic activity and no induction of micronuclei was reported in an *in vitro* mammalian cell micronucleus test conducted in agreement with OECD 487. No increases in the micronucleated polychromatic erythrocytes were found in the *in vivo* micronucleus test in rat bone marrow conducted according to OECD Guideline 474, but no evidence of target exposure was provided. Finally, no treatment-related adverse effects were reported in the sub-chronic oral toxicity test performed in rats conducted in compliance with OECD Guideline 408.

3.2.4. Safety for the user

The tests under this section were done with the liquid intermediate used to formulate the additive and not with the final formulations.

3.2.4.1. Effects on the respiratory system

The test item was assessed in an acute inhalation toxicity study in rats exposed to a mean achieved concentration of 5.02 mg/L for four hours, in accordance with OECD Guideline 403. No rats died during the observation period, the signs observed regarding the respiration of the rats (laboured respiration, increased respiratory rate, noisy respiration and weak condition) disappeared from day 5, body weights were not changed and the necropsy revealed no changes in the treated animals. Therefore, under the experimental conditions used the test item showed no respiratory toxicity.

Owing to the proteinaceous nature of the active substances of the additive, it is considered a potential respiratory sensitiser.

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28 Technical dossier/Section III/Annex III.8.
29 Technical dossier/Section III/Annex III.9.
30 Technical dossier/Section III/Annex III.10.
31 Technical dossier/Section III/Annex III.12.
32 Technical dossier/Section III/Annex III.14.
3.2.4.2. Effects on skin and eyes

The acute dermal irritant potential of the enzyme concentrate was investigated in three New Zealand White rabbits in accordance with OECD Guideline 404. From the results the test item is not irritant to skin.

The acute eye irritation potential of the enzyme concentrate was investigated in three New Zealand White rabbits in accordance with OECD Guideline 405. From the results it is concluded that the test item is not irritant to eyes.

No data were provided regarding the dermal sensitisation potential of the test item.

3.2.4.3. Conclusions on safety for the user

The studies under this section were performed with an intermediate product and not with the final formulations. The intermediate product did not prove to be toxic by inhalation or irritant for skin or eye and the ingredients used to formulate the additive are not likely to contribute to the irritant properties. Owing to the lack of data, the Panel cannot conclude on the dermal sensitisation potential of the additive. Because of the proteinaceous nature of the active substance, the additive is considered a potential respiratory sensitiser.

3.2.5. Safety for the environment

The active substance of the additive is a protein, and as such will be degraded/inactivated during passage through the digestive tract of animals or in the environment. The production strain was not detected in the concentrate and therefore it would not be detected in the additive. Although the presence of DNA was shown in the enzyme concentrate, this is not considered a safety concern. Therefore, the Panel concludes that the additive does not raise concerns regarding the environment.

3.3. Efficacy

3.3.1. Efficacy for chickens for fattening

The applicant submitted four trials with chickens for fattening. The details on the study design are provided in Table 1 and the main results in Table 2. In all trials one-day-old birds were used, in trials 1, 2 and 4 males were used while in trial 3 males and females were used and kept sex separated. In all four studies the birds were fed either a non-supplemented diet (control) or a diet containing the Beltherm xylanase at different levels from 50 to 200 ADXU/kg (up to 20,000 ADXU/kg feed in trial 2) (confirmed by analysis). All studies included the recommended dose of 100 ADXU/kg feed. The diets were administered from day 1 of life and for at least 36 days. The health and mortality were monitored throughout the study and the body weight and feed intake were recorded. Feed to gain ratio was calculated. The data was analysed with an ANOVA, using the pen as the experimental unit. Group means were compared with Tukey test in trial 1 and 4, Dunnett test in trial 2 and Student Newman Keuls test in trial 3.

The results of the individual studies showed that feed to gain ratio was better in birds receiving Beltherm at 200 ADXU/kg feed in two trials (1 and 4) and at 100 ADXU/kg in one trial (trial 2). Similarly, final weight was significantly improved in two trials at 200 ADXU/kg feed (trial 2 and 4).

Data from all four studies pooled and analysed statistically in order to study the effect of the additive when added at the dose of 100 ADXU/kg feed. Only the data from males was included in the study, therefore, the data on the females from trial 3 was not considered. Parameters studied included feed intake, final body weight and feed to gain ratio. The model used considered the effect of treatment, the trial and their interaction and the initial body weight of the birds as a covariable. No interactions between treatment and trial were revealed. The results showed that the animals receiving the additive at 100 ADXU/kg feed were heavier at the end of the trial (2,407 vs 2,463 g P = 0.04) and had a higher average daily weight gain (61.1 vs 62.5 g) compared to the non-supplemented group. No differences were found in the other parameters investigated.

33 Technical dossier/Section III/Annex III.15.
34 Technical dossier/Section III/Annex III.16.
Table 1: Trial design and dosages of the efficacy trials performed in chickens for fattening

| Trial | Total No of animals (animals x replicate x treatment) | Breed sex (duration) | Composition feed (Form) | Enzyme activity (ADXU/kg feed) |
|-------|-----------------------------------------------------|----------------------|-------------------------|-------------------------------|
|       |                                                     |                      |                         | Intended                      |
| 135   | 960 (30)                                            | Ross 308 Males (39 d)| Wheat, soya bean meal (Mash) | 0                             |
|       | 8                                                   |                      |                         | 50, 53, 50, 36                |
|       |                                                     |                      |                         | 100, 93, 91, 92               |
|       |                                                     |                      |                         | 200, 206, 172, 198            |
| 236   | 2,208 (46)                                          | Ross PM3 Males (36 d)| Wheat, soya bean meal, maize and rye (pellet) | 0                             |
|       | 12                                                  |                      |                         | 100, 103, 138                 |
|       |                                                     |                      |                         | 200, 223, 254                 |
|       |                                                     |                      |                         | 20,000, 26,099, 22,986        |
| 337   | 680 (17)                                            | Ross 308 Males and females (42 d)| Wheat, soya bean meal (pellet) | 0                             |
|       | 10                                                  |                      |                         | 50, 43, 51                    |
|       |                                                     |                      |                         | 100, 88, 101                  |
|       |                                                     |                      |                         | 200, 159, 189                 |
| 438   | 900 (30)                                            | Ross 308 Males (39 d)| Wheat, rye and soya bean meal (mash) | 0                             |
|       | 10                                                  |                      |                         | 100, 96, 77, 84               |
|       |                                                     |                      |                         | 200, 178, 177, 178            |

Table 2: Effects of Beltherm on the performance of chickens for fattening

| Trial | Enzyme activity (ADXU/kg feed) | Feed intake (g)(1) | Final body weight (g) | Feed to gain ratio | Mortality and culling (%) |
|-------|---------------------------------|--------------------|-----------------------|--------------------|----------------------------|
| 1     | 0                               | 103                | 2,414                 | 1.68<sup>a</sup>   | 6.7                        |
|       | 50                              | 104                | 2,466                 | 1.67<sup>ab</sup>  | 5.0                        |
|       | 100                             | 105                | 2,522                 | 1.66<sup>ab</sup>  | 4.6                        |
|       | 200                             | 104                | 2,517                 | 1.64<sup>b</sup>   | 3.7                        |
| 2     | 0                               | 3,703              | 2,261                 | 1.66               | 3.4                        |
|       | 100                             | 3,589              | 2,311                 | 1.57<sup>*</sup>   | 6.7                        |
|       | 200                             | 3,708              | 2,355<sup>*</sup>      | 1.59<sup>*</sup>    | 4.5                        |
|       | 20,000                          | 3,469<sup>*</sup>   | 2,328<sup>*</sup>      | 1.50<sup>*</sup>    | 6.3                        |
| 3     | 0                               | 95                 | 2,410                 | 1.69               | 1.8                        |
|       | 50                              | 92                 | 2,407                 | 1.66               | 2.3                        |
|       | 100                             | 93                 | 2,416                 | 1.66               | 0.6                        |
|       | 200                             | 90                 | 2,367                 | 1.65               | 0.0                        |
| 4     | 0                               | 99<sup>a</sup>     | 2,386<sup>a</sup>     | 1.64<sup>a</sup>   | 3.7                        |
|       | 100                             | 99<sup>b</sup>     | 2,417<sup>b</sup>     | 1.62<sup>ab</sup>  | 4.7                        |
|       | 200                             | 104<sup>b</sup>    | 2,557<sup>b</sup>     | 1.61<sup>b</sup>   | 4.7                        |

<sup>(1)</sup> Values in trials 1, 2 and 4 are daily feed intake and in trial 2 total feed intake.
<sup>*</sup> Values are significantly different compared to the control group P < 0.05.
<sup>a,b</sup> Mean values within a trial and within a column with a different superscript are significantly different P < 0.05.

In only one of the individual studies the dose of 100 ADXU/kg feed showed a positive response on the feed to gain ratio of the birds. However, based on the results of the statistical analysis of the data pooled from the four efficacy trials the Panel concludes that the additive has a potential to improve the performance of chickens for fattening at the recommended dose (100 ADXU/kg feed).

3.3.2. Efficacy for turkeys for fattening

The applicant provided four trials and a statistical analysis that pooled the data from the four trials. One of the trials was not considered further due to the high mortality and culling registered (mean...
value 14.0 %). The statistical analysis of the pooled data included the data from this study and therefore was not considered.

The details of the study design for the three trials considered are provided in Table 3 and the main results in Table 4. In all trials one-day-old birds were used, in trials 1 and 2, males were used and in trial 3, females. In the three studies the birds were fed either a non-supplemented diet (control) or a diet containing the Beltherm xylanase at 100 or 200 ADXU/kg feed (see analysed values in Table 3). The feeds were administered from day 1 of life for 112 days in trials 1 and 2 and for 84 days in trial 3. The health and mortality were monitored and the body weight and feed intake were measured throughout the study. Feed to gain ratio was calculated. The data were analysed with an ANOVA, using the pen as the experimental unit and group means were compared with Least Significant Difference test in trial 1, Tukey Test in trial 2 and Duncan Test in trial 3.

Table 3: Trial design and dosages of the efficacy trials performed in turkeys for fattening

| Trial | Total No of animals (Animals x replicate Replicates x treatment) | Breed Sex (duration) | Composition feed (form) | Enzyme activity (ADXU/kg feed) |
|-------|---------------------------------------------------------------|----------------------|-------------------------|--------------------------------|
| 1     | BUT T9 Males (112 d)                                          | Wheat, rye soya bean meal (mash/pellet) | 0                       |                                |
|       | (28)                                                          |                      | 100                     | 221, 212, 186, 239             |
|       | 8                                                             |                      | 103, 96, 86, 114        |                                |
| 2     | BUT T9 Males (112 d)                                          | Wheat, soya bean meal (mash/pellet) | 0                       |                                |
|       | (28)                                                          |                      | 100                     | 189, 111, 210, 197             |
|       | 8                                                             |                      | 69, 51, 72, 97          |                                |
| 3     | BUT 10 Females (84 d)                                         | Wheat, soya bean meal (pellets) | 0                       |                                |
|       | (25)                                                          |                      | 100                     | 53, 55, 49                    |
|       | 8                                                             |                      | 108, 118, 141           |                                |

Table 4: Effects of Beltherm on the performance of turkeys for fattening

| Trial | Diets | Daily feed intake (g) | Final body weight (g) | Feed to gain ratio | Mortality and culling (%) |
|-------|-------|-----------------------|-----------------------|--------------------|----------------------------|
| 1     | 0     | 274                   | 13,240<sup>a</sup>    | 2.32               | 8.5                        |
|       | 100   | 283                   | 13,830<sup>a</sup>    | 2.30               | 9.0                        |
|       | 200   | 281                   | 13,644<sup>ab</sup>   | 2.32               | 8.5                        |
| 2     | 0     | 270<sup>b</sup>       | 13,724                | 2.21               | 4.8                        |
|       | 100   | 271<sup>ab</sup>      | 13,777                | 2.21               | 6.8                        |
|       | 200   | 263<sup>b</sup>       | 13,490                | 2.19               | 6.8                        |
| 3     | 0     | 173                   | 6,903                 | 2.13<sup>a</sup>    | 3.5                        |
|       | 100   | 175                   | 7,025                 | 2.11<sup>ab</sup>   | 4.0                        |
|       | 200   | 170                   | 6,939                 | 2.08<sup>b</sup>    | 4.5                        |

<sup>a,b</sup> Mean values within a trial and within a column with a different superscript are significantly different P < 0.05.

Turkeys receiving Beltherm at 100 ADXU/kg feed were significant heavier in one trial (trial 1), and those receiving Beltherm at 200 ADXU/kg (analysed activity similar to 100 ADXU/kg feed) showed a better feed to gain ratio in another trial (trial 3). It is noted that in trial 1, the comparison of the group means was performed with LSD test which does not correct for multiple comparisons and since there were three groups such correction would be desired when comparing the group means.

Based on the data provided, the Panel cannot conclude on the efficacy of Beltherm in turkeys for fattening.

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39 Technical dossier/Section IV/Annex IV.03.3 and Supplementary information February 2017.
40 Technical dossier/Section IV/Annex IV.03.1 and Supplementary information February 2017/Annex Q27a.
41 Technical dossier/Section IV/Annex IV.03.2 and Supplementary information February 2017/Annex Q27b.
42 Technical dossier/Section IV/Annex IV.03.4 and supplementary information February 2017/Annex Q27d.
3.3.3. Efficacy for turkeys for breeding purposes

No data supporting the efficacy of the product in turkeys for breeding purposes were submitted. Therefore, the Panel cannot conclude on the efficacy of the additive in turkeys for breeding purposes.

3.3.3.1. Conclusions on efficacy

The FEEDAP Panel concludes that the additive has the potential to improve the performance of chickens for fattening at the dose of 100 ADXU/kg feed. This conclusion is extended to chickens reared for laying. The mode of action of xylanases is well known and considered to be similar among poultry species, therefore the conclusion can be extrapolated to minor growing poultry species.

Due to the limited evidence provided in turkeys for fattening and the lack of data in turkeys for breeding purposes the Panel cannot conclude on the efficacy of the product in turkeys for fattening or reared for breeding nor in turkeys for breeding purposes.

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\(^{43}\) and Good Manufacturing Practice.

4. Conclusions

The recipient strain belongs to a species that is considered by EFSA to qualify for the QPS approach to safety assessment. The strain was unequivocally identified, qualifications were met and the genetic modification raised no concerns. The production strain was not detected in the enzyme concentrate and therefore would not be detected in the additive. Although the presence of DNA was shown in the enzyme concentrate, this is not considered a safety concern.

The additive is safe for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, turkeys for breeding purposes and minor poultry species at 100 ADXU per kg feed with a wide margin of safety.

The additive is safe for the consumers of food derived from animals fed with the additive.

The additive, in either form, is not toxic by inhalation or irritant for skin or eye but it is considered a potential respiratory sensitizer. The Panel cannot conclude on the dermal sensitisation potential of the additive.

The use of the product as a feed additive is of no concern for the environment.

The additive has the potential to improve the performance of chickens for fattening at the dose of 100 ADXU/kg feed. The conclusion is extended to chickens reared for laying and extrapolated to minor growing poultry species. The Panel considers that there is insufficient data to conclude on the efficacy of the product in turkeys for fattening, turkeys reared for breeding or in turkeys for breeding purposes.

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\(^{43}\) 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.
Documentation provided to EFSA

1. Beltherm MP/ML for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, minor poultry species and turkeys for breeding purposes. February 2017. Submitted by Puratos.

2. Beltherm MP/ML for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, minor poultry species and turkeys for breeding purposes. Supplementary information. February 2017. Submitted by Puratos.

3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Beltherm MP/BL.

4. Comments from Member States.

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Abbreviations

ANOVA     analysis of variance
CFU       Colony forming unit
CV        Coefficient of variance
EC        European Commission
EURL      European Union Reference Laboratory
MIC       Minimum inhibition concentration
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Beltherm

In the current application authorisation is sought for Beltherm® MP &ML under article 4(1) (new feed additive) under the category/functional 4(a) "zootechnical additives"/"digestibility enhancers". Specifically, authorisation is sought for the use of the feed additive for chickens for fattening and reared for laying, turkeys for fattening, for breeding and reared for breeding and minor poultry species. According to the Applicant, endo 1,4-beta-xylanase is the active substance of Beltherm produced by a genetically modified strain of the bacteria Bacillus subtilis. The Applicant expresses the xylanase enzymatic activity in ADXU units defined as "the amount of enzyme which liberates one micromole of reducing sugars (xylose equivalent) per minute from beechwood xylan at pH 6.0 and 70 °C". The product is intended to be marketed as a granulated and liquid formulations (MP and ML) having a guaranteed minimum xylanase activity of 500 ADXU/g. The carrier in the solid formulation is wheat flour, while glycerol in acetate buffer is used for the liquid formulation. The feed additive is intended to be included through premixtures or directly in feedingstuffs to obtain a minimum activity of 100 ADXU/kg of feedingstuffs for all the target species.

For the quantification of the xylanase activity in the feed additive the Applicant proposed a single-laboratory validated and further verified colorimetric method based on the measurement of reducing sugars (xylose equivalents) released by the action of xylanase on 3% beechwood xylan substrate in the presence of 3.5-dinitrosalicylic acid (DNS) at pH 6.0 and 70 °C (DNS method).

For the quantification of the xylanase activity in premixtures and feedingstuffs, the Applicant proposed another single-laboratory validated and further verified colorimetric method, based on the quantification of water soluble dyed fragments produced by the action of xylanase on commercially available azurine cross-linked arabinoxylan substrates.

Based on the satisfactory performance characteristics, the EU RL recommends for official control the proposed single-laboratory validated and further verified colorimetric methods for the quantification of the xylanase activity in the feed additive (DNS method) premixtures and feedingstuffs.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.