SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 25 January 2017

doi: 10.2903/j.efsa.2017.4707

Safety and efficacy of Bergazym® P100 (endo-1,4-β-xylanase) as a feed additive for chickens for fattening, weaned piglets and pigs for fattening

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Guido Rychen, Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace, Pieter Wester, Paul Brantom, Noël Albert Dierick and Montserrat Anguita

Abstract

Bergazym® P is a preparation of endo-1,4-β-xylanase (xylanase) that is intended to be used as a zootechnical additive in feed for chickens for fattening, weaned piglets and pigs for fattening at a dose of 1,500 EPU/kg feed. The xylanase is produced by a non-genetically modified strain of *Trichoderma reesei*. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded from the tolerance studies submitted that the additive is safe for chickens for fattening and weaned piglets at 1,500 EPU/kg feed. The conclusions were extended to pigs for fattening. The results obtained with the enzyme concentrate used to formulate the additive in the genotoxicity studies and in the sub-chronic oral toxicity study do not indicate any reason for concern for consumer safety arising from the use of the product as feed additive. From the data provided on the enzyme concentrate used to formulate the additive, the Panel concluded that the additive is not irritant to skin or eyes but it is considered a potential skin sensitiser. Owing to the proteinaceous nature of the active substance, the additive is considered a potential respiratory sensitiser. The FEEDAP Panel concluded that no risks to the environment are expected. To support the efficacy of the additive, the applicant submitted five trials in chickens for fattening, five in weaned piglets and three in pigs for fattening. The FEEDAP Panel could not conclude on the efficacy of the additive in chickens for fattening and weaned piglets, but concluded that the additive has the potential to be efficacious in pigs for fattening at the recommended dose.

Keywords: zootechnical additives, digestibility enhancers, xylanase, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2014-00586

Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti Giovanna, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Dierick NA and Anguita M, 2017. Scientific opinion on the safety and efficacy of Bergazym® P100 (endo-1,4-β-xylanase) as a feed additive for chickens for fattening, weaned piglets and pigs for fattening. EFSA Journal 2017;15(2):4707, 15 pp. doi:10.2903/j.efsa.2017.4707

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

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 Bergazym® P100 (endo-1,4-β-xylanase) as a feed additive for chickens for fattening, weaned piglets and pigs for fattening.

Bergazym® P100 is a feed additive with endo-1,4-β-xylanase (xylanase) as the main enzyme activity which is available in solid form. The xylanase is produced by a non-genetically modified strain of *Trichoderma reesei*.

The results found in the tolerance trials provided in chickens for fattening and weaned piglets showed that the animals can tolerate 200- or 100-fold the recommended dose of 1,500 EPU/kg feed, respectively. Therefore, the FEEDAP Panel concluded that the additive is safe for these target species/categories. The conclusions reached for the weaned piglets were extended to pigs for fattening.

The enzyme concentrate used to formulate the additive was tested in genotoxicity studies and in a sub-chronic oral toxicity study. The results of these tests did not indicate any reason for concern for consumer safety arising from the use of the product as feed additive. Therefore, the Panel concluded that the additive is safe for the consumers.

The enzyme concentrate did not prove irritant for skin or eye but proved positive in the skin sensitising test. The Panel considered that the ingredients used to formulate the additive are not likely to contribute to the irritant properties and therefore concluded that the additive is not irritant to skin and eyes but is considered a potential skin and respiratory sensitiser.

The FEEDAP Panel concluded that the use of Bergazym® P100 as a feed additive poses no risks to the environment.

To support the efficacy of the additive, the applicant submitted five trials in chickens for fattening, five in weaned piglets and three in pigs for fattening. From these studies the Panel identified the need for further studies in order to conclude on the efficacy of the additive in chickens for fattening and weaned piglets. The Panel concluded that the additive has the potential to be efficacious in pigs for fattening at the recommended dose.

Table of contents

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 Berg + Schmidt GmbH Co. KG[2] for authorisation of the product Bergazym® P100 (endo-1,4-β-xylanase), when used as a feed additive for chickens for fattening, weaned piglets and pigs 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). The particulars and documents in support of the application were considered valid by EFSA as of 24 June 2015.

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 Bergazym® P100 (endo-1,4-β-xylanase), when used under the proposed conditions of use (see Section 3.1.5).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[3] in support of the authorisation request for the use of Bergazym® P100 as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[4] 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]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Bergazym® P100 is in line with the principles laid down in Regulation (EC) No 429/2008[4] 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), 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), Technical Guidance: Microbial Studies (EFSA, 2008a), Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008b, revised in 2009) and Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008c).

3. Assessment

Bergazym® P100 (endo-1,4-β-xylanase; xylanase (Enzyme Commission number: 3.2.1.8)) is intended to be used as a zootechnical additive, functional group of digestibility enhancers, for chickens for fattening, weaned piglets and pigs for fattening.

[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] Berg + Schmidt GmbH Co. KG, An der Alster 81, 20099 Hamburg, Denmark.
[3] FEED dossier reference: FAD-2014-0029.
[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/default/files/FinRep-FAD-2014-0029.pdf

3.1. Characterisation

3.1.1. Characterisation of the active substance

Bergazym® P100 contains a xylanase produced by a non-genetically modified strain of *Trichoderma reesei* deposited at the Belgian Coordinated Collections of Microorganisms with the accession number BCCM/MUCL 49755 (for an undefined period, dated 2011).[6] The additive also contains cellulase and β-glucanase as side activities.[7]

3.1.2. Manufacturing process[8]

The enzyme is produced in a multistep process including fermentation, filtration and concentration. The resulting product is formulated with wheat meal and starch. The applicant stated that no antimicrobial substances are used in the manufacture of the product.

3.1.3. Characterisation of the additive

The additive is available in a coated granular form which contains 2.6% of enzyme concentrate, wheat meal (96.7%) and starch (0.7%).[9] The additive is specified to a minimum activity of 15,000 EPU[10] /g. The study of the batch to batch variation in six batches showed a mean enzyme activity of 15,317 EPU/g with a coefficient of variation (CV) of 3.3%.[11]

Three to four batches of the additive were analysed for microbiological contamination and chemical contamination.[12] The analyses of chemical contamination included arsenic (< 0.5 mg/kg), cadmium (< 0.5 mg/kg), lead (< 1.5 mg/kg), mercury (< 0.02 mg/kg), fluorine (< 5 mg/kg, only one batch), polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) (WHO-PCDD/F-TEQ ≤ 0.184 ng/kg), dioxin-like PCBs (WHO-PCB-TEQ ≤ 0.067 μg/kg) and sum of PCDD and PCDF and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ ≤ 0.250 ng/kg). Microbiological analysis included *Salmonella* spp. (absent in 25 g), *Escherichia coli* (< 10 colony forming units (CFU)/g), coliforms (≤ 30 CFU/g), total viable aerobic counts (≤ 10^3 CFU/g, two batches), yeasts and moulds (≤ 10^2 CFU/g, two batches). The absence of the production strain was demonstrated in one further batch.[13] The following mycotoxins were also analysed: aflatoxin B1, B2, G1 and G2 (< 1.7 μg/kg), zearalenone (< 17 μg/kg), deoxynivalenol (< 40 μg/kg),[14] ochratoxin A (< 21 μg/kg, two batches), fumonisin (< 47 μg/kg, two batches) and citrinin (< 111 μg/kg, two batches). The presence of other secondary metabolites was investigated[14]; trichodermins (below limit of detection (LOD) 4 μg/kg), trichodimerols (below LOD 2 μg/kg), sorbicillactones (below LOD 800 μg/kg). One batch of the product was investigated for the presence of antimicrobial activity. The results showed absence of antimicrobial activity (according to EFSA, 2008a).[15]

Particle size was studied in three batches by laser diffraction and showed that particles below 100 μm amount ~ 2.4%, below 52 μm amount ~ 1.3% and below 18 μm amount 0.5% (v/v).[16] Dusting potential of three batches, measured following the Stauber–Heubach method, ranged from 0 to 0.02 g/m^3).[17]

3.1.4. Stability and homogeneity

The shelf-life of the additive was investigated in three batches. Samples were stored in closed plastic bags for 6 months at three different temperatures, 25, 30 or 40°C.[17] After 6 months, the mean enzyme recoveries of the initial enzyme activity were 90% at 25°C, 84% at 30°C and 60% at

[6] Technical dossier/Supplementary information November 2016/Annexes 1.1, 1.4 and 2. Deposition for an undefined period, letter dated 2011.
[7] Technical dossier/Supplementary information November 2016/Annexes 7.1 to 7.3.
[8] This section has been amended following the confidentiality claims made by the applicant.
[9] Technical dossier/Supplementary information November 2016.
[10] Technical dossier/Section II/Annex 2.6.1./One EPU, endopentosanase unit, is defined as the amount of enzyme required to release 0.0083 μmol of reducing sugar equivalents (xylose equivalents) from oat spelt xylan per minute at pH 4.7 and 50°C.
[11] Technical dossier/Section II/Annexes 2.1.3.a and 2.1.3.b.
[12] Technical dossier/Section II/Annexes 2.1.4.a and 2.1.4.b and Supplementary information November 2016/Annex 9.
[13] Technical dossier/Supplementary information November 2016/Annex 11.
[14] Technical dossier/Supplementary information November 2016/Annex 4.
[15] Technical dossier/Supplementary information November 2016/Annex 10.
[16] Technical dossier/Section II/Annex 2.1.5.
[17] Technical dossier/Section II/Annex 2.1.3.b.

40°C.Three batches of the additive were incorporated to a vitamin–mineral premixture for poultry feed (without choline chloride) at a dose of 200 EPU/g.[17] Samples were kept in closed plastic bags for 3 and 6 months at 25°C. Mean recoveries of the initial enzyme activity after 3 and 6 months of storage were 75% and 79%, respectively.

The stability to pelleting was measured by incorporating three batches of the additive to a complete feed for poultry species at a dose of 2,400 EPU/kg.[17] The mash feed was pelleted and the enzyme activities were measured in samples taken after conditioning at 75°C and after pelleting (temperature in the outlet 81°C). The mean recoveries of the initial enzyme activity at these two points were 84% and 49%, respectively, indicating low stability at those temperatures. In a further test at 68°C,[18] the recovery was 95%.

The stability in mash feed and pelleted feed was studied in complete feed for chickens for fattening in mash (two different studies) or pelleted form supplemented at 2,000 EPU/kg feed.[19] Samples were stored at 25°C for 3 months. In a first study in mash feed, mean recovery of the initial enzyme activity after 3 months was 62% and in the second study no loss of activity was found. In pelleted feed, no loss of activity was found after 3 months.

The capacity of the additive to homogeneously distribute in feed was studied in 10 sub-samples of four batches of mash feed and in two batches of pelleted feed.[20] In the mash feed the CV values ranged from 12% to 56%, indicating low capacity to distribute homogeneously. I n the pelleted feed the values were below 10%.

3.1.5. Conditions of use

Bergazym® P100 is intended to be used as a feed additive for chickens for fattening, weaned piglets and pigs for fattening at a recommended dose of 1,500 EPU/kg feed.

3.2. Safety

3.2.1. Safety for the target species

3.2.1.1. Safety for chickens for fattening

Two tolerance trials were provided. One of them[21] was not considered further in the assessment because the animals were not healthy; the birds suffered from an episode of necrotic enteritis for which they had to be medically treated (4 days of antibiotic) and which resulted in a high mortality/culling in all treatments (mean 14.5%).

In the second study,[22] a total of 144 one-day-old female Ross 308 chickens were distributed to 24 wire-floor cages in groups of six chickens each and allocated to three dietary treatments (representing eight replicate cages per treatment). Basal diets (starter and grower) based on wheat, rye and soybean were either not supplemented (control) or supplemented with Bergazym® P100 to provide 1,500 (1×) or 300,000 EPU/kg feed (200×). The enzyme activity was confirmed by analysis. Feed and water were available *ad libitum* over an experimental period of 35 days. General health status and mortality were monitored daily. Body weight and feed intake were recorded at days 1, 14 and 35, and feed to gain ratio calculated. An analysis of variance (ANOVA) was performed with the data considering the cage was the experimental unit. Group means were compared with the least significant difference test. Significance level was set at $p < 0.05$.

Five birds died during the study, two in the control, two in the 1× and one in the 200×. Daily feed intake of the birds was 97.8, 96.6 and 93.6 g/day in the control, 1× and 200×, respectively. The corresponding figures for body weight were 2,053, 2,106 and 2,147 g and for feed to gain ratio were 1.70, 1.64 and 1.56. The 200-fold supplementation did not adversely affect the health and performance of the birds. Therefore, the FEEDAP Panel concludes that the additive is safe for chickens for fattening at the recommended dose.

[18] Technical dossier/Section II/Annex 2.4.1.a.
[19] Technical dossier/Section II/Annex 2.1.3.b, Annex 2.4.1.a and Annex 2.4.1.b.
[20] Technical dossier/Section II/Annex 2.1.3.b, Annex 2.4.2. and Supplementary information November 2016/Annex 16.
[21] Technical dossier/Section III/Annex 3.1.1.e 14-21 Final report, Annex 3.1.1.f, Annex 3.1.1.g, and Annex 3.1.1.h.
[22] Technical dossier/Supplementary information November 2016/Annex 18.1.a–d.

3.2.1.2. Safety for weaned piglets

Two tolerance trials were provided. One of them[23] was not considered further due to the extensive treatment with antibiotics administered to the piglets (6–8 days via feed).

In the second tolerance trial,[24] a total of 135 weaned male and female piglets (Landrace × Large White, initial body weight 5.01 kg) were distributed to pens in groups of five piglets each (mixed gender) and allocated to three dietary treatments (representing nine replicate pens per treatment). Basal diets (pre-starter and starter) based on wheat, barley, rye and soybean were either not supplemented (control) or supplemented with Bergazym® P100 to provide 1,500 (1×) or 150,000 (100×) EPU/kg feed. The enzyme activity in the feed was confirmed by analysis. Feed and water were available *ad libitum* over an experimental period of 42 days. General health status and mortality were monitored daily. Body weight and feed intake were recorded at days 1, 14 and 42, and average daily gain, average daily feed intake and feed to gain ratio calculated. An ANOVA was performed with the data considering the pen as the experimental unit. Significance level was set at $p < 0.05$.

Five animals died during the study (mean mortality value of 4.5%); four piglets died in the 1× dose (two due to digestive disorders, one with meningitis and another one with septicaemia) and one piglet died in the 100× (at the pre-starter phase with signs of anorexia). Three more piglets were removed from the study, two from 1× (low performer, meningitis) and one from 100× (low performer). No animals died or were culled in the control, the mortality/culling registered in the 1× dose was 13% and in the 100× was 5.7%. The daily feed intake was 425, 423 and 422 g/day for control, 1× and 100×, respectively. The corresponding figures for final body weight were 17.4, 17.5 and 17.6 kg and the feed to gain ratio was 1.44, 1.42 and 1.41. There were no significant effects of the addition of Bergazym® P100 on the performance parameters considered.

The group receiving the recommended dose showed a high mortality/culling rate. Considering the likely cause of death reported, that no other negative effects on the performance of the piglets in the recommended dose group were identified and that this high mortality rate was not found in the piglets receiving 100×, the FEEDAP Panel considers that this mortality is not related to the dietary treatment. Therefore, the FEEDAP Panel concludes that the additive is safe for the weaned piglets at the recommended dose.

3.2.1.3. Safety for pigs for fattening

No specific study was provided to demonstrate the tolerance of pigs for fattening. The FEEDAP Panel considers that the conclusions reached with regard to weaned piglets can be extended to pigs for fattening.

3.2.1.4. Conclusions on the safety for the target species

The results from the tolerance trials provided in chickens for fattening and weaned piglets showed that the animals can tolerate 200 or 100-fold the recommended dose of 1,500 EPU/kg feed, respectively. Therefore, the FEEDAP Panel concludes that the additive is safe for these target species at the recommended dose. The conclusions reached for the weaned piglets can be extended to pigs for fattening.

3.2.2. Safety for the consumer

In the studies presented below, the test item was the enzyme concentrate used to formulate the additive.[9]

3.2.2.1. Genotoxicity studies

Bacterial reverse mutation assay

The test substance diluted in dimethylsulphoxide was examined for mutagenic activity in the bacterial reverse mutation test using the *Salmonella* Typhimurium strains TA 1535, TA 1537, TA 98, TA 100 and the *Escherichia coli* strain WP2 uvrA, in the absence and presence of a liver fraction of Aroclor 1254-induced rats for metabolic activation (S9-mix) according to OECD Guideline 471.[25] The maximum concentration was 5,000 μg/plate. The test item did not induce any dose-related or more than twofold

[23] Technical dossier/Section III/Annex 3.1.1.a–d.
[24] Technical dossier/Supplementary information November 2016/Annex 16.a–b.
[25] Technical dossier/Section III/Annex 3.2.2.a.

increase in the number of revertant colonies, whereas the positive controls gave the expected mutagenic effect.

In vitro chromosomal aberration test

The test substance, diluted in dimethylsulphoxide, was examined for its potential to induce structural chromosomal aberrations in Chinese hamster ovary cells, in both the absence and presence of a metabolic activation system (S9-mix) in compliance with OECD Guideline 473 (rev.1997).[26] Two separate tests were conducted. In the first test, the treatment/harvesting times were 4/18 h (pulse treatment) both in the absence and in the presence of S9-mix. In the second test, a pulse treatment was applied in the presence of S9-mix and a continuous treatment of 18 h, directly followed by harvesting, in the absence of S9-mix. In all instances, duplicate cultures were used.

The highest concentration tested was based on the solubility of the test substance in the culture medium. In the pulse treatment, slight cytotoxicity was reported at the two highest concentrations of 1,500 and 1,000 μg/mL; in the continuous treatment, clear cytotoxicity was reported at the two highest concentrations of 600 and 400 μg/mL. The test substance did not induce any statistically significant increase in the number of aberrant cells in any experimental condition, whereas the positive control performed as expected.

3.2.2.2. Sub-chronic oral toxicity study

The study was conducted in accordance with OECD guideline 408.[27] The trial was carried out over 13 weeks in groups of 10 Wistar rats of each sex, caged in groups of five. There was a total of seven experimental groups, with one negative control, three groups given diets containing the test item at 500, 1,500 and 4,000 mg/kg body weight and day. The other three groups are not relevant to the current assessment. The enzyme activity of the experimental diets was confirmed by analysis. There were no significant adverse effects of treatment on any of the parameters observed during or at the end of the experimental period.

3.2.2.3. Conclusions on the safety for the consumer

The results obtained with the enzyme concentrate used to formulate the additive in the genotoxicity studies and in the sub-chronic oral toxicity study do not indicate any reason for concern for consumer safety arising from the use of the product as feed additive.

3.2.3. Safety for the user

In the studies presented below, the test item was the enzyme concentrate used to formulate the additive.[9]

3.2.3.1. Respiratory system

No specific studies were provided to address the effects of the additive on the respiratory system. Owing to the proteinaceous nature of the active substance, the additive is considered as a potential respiratory sensitiser. However, the inhalation exposure is expected to be low because the dusting potential of the product is negligible.

3.2.3.2. Skin and eyes

The irritant properties of the fermentation product to skin and eyes were evaluated in rabbits according to OECD test Guidelines 404 and 405, respectively.[28] The test item was classified as not irritant.

The skin sensitising potential of the fermentation product was evaluated in the local lymph node assay in mouse following OECD test Guideline 429.[29] No signs of irritancy were found. From the results obtained, the test item should be regarded as a skin sensitiser.

3.2.3.3. Conclusions on safety for the user

The enzyme concentrate is not irritant to skin or eye but is a skin sensitiser. The ingredients used to formulate the additive are not likely to contribute to the irritant properties and therefore the

[26] Technical dossier/Section III/Annex 3.2.2.b.
[27] Technical dossier/Section III/Annexes 3.2.2.3a–c.
[28] Technical dossier/Section III/Annexes 3.3.1.2.b and 3.3.1.2.c.
[29] Technical dossier/Section III/Annex 3.3.1.2.a.

additive is not irritant to skin and eyes. The additive is considered a potential skin and respiratory sensitiser.

3.2.4. 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. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

3.3. Efficacy

3.3.1. Efficacy in chickens for fattening

Five long-term trials in chickens for fattening were submitted. Two of these studies were not considered further. One[30] was not considered further due to the unavailability of relevant data/information including methodologies followed, analytical data of the feed offered and data regarding the animals. The other study was the first tolerance study described above (see Section 3.2.1.1)[31] which could not be considered because the birds were not healthy.

Details of the design of the three studies considered are provided in Table 1 and the results in Table 2. The third study is the second tolerance study presented in Section 3.2.1.1. In all trials, 1-day-old birds were used and at least two experimental groups were considered, a control group and a group receiving the recommended dose. Enzyme dosage was not confirmed in study 1. The health of the animals and mortality were monitored throughout the study and the body weight and feed intake were recorded. Feed to gain ratio was calculated. In the first trial there were measurements on the utilization of the diets, however, these data did not include the measurement of the energy and therefore it is not presented below. An ANOVA was performed with the data obtained considering the pen as the experimental unit. The comparison of the mean groups was performed with the Tukey test in trial 1 and with the least significant difference test in trials 2 and 3. Significance level was set at $p < 0.05$.

Table 1: Trial design and dosages of the efficacy trials performed in chickens for fattening

Trial	Total no. animals (animals/replicate) Replicates/treatment	Breed Duration Sex	Diet composition of the diets	Enzyme activity (EPU/kg feed)	
				Intended	Analysed
1[(a)]	600 (50) 4	Cobb 500 49 ♂/♀	Maize, soya bean meal	0 1,500 3,000	Not provided
2[(b)]	96 (6) 8	Ross 308 35 ♀	Wheat, rye, barley, soya bean meal	0 1,500	390 1,285
3[(C)]	144 (6) 8	Ross 308 35 ♀	Wheat, rye, soya bean meal	0 1,500 300,000	388 1,365 446,000

(a): Technical dossier/Section IV/Annex 4.3.1.2.a–b. and Supplementary information November 2016/Annex 19.
(b): Technical dossier/Supplementary information November 2016/Annex 18.2.a–d.
(c): Technical dossier/Supplementary information November 2016/Annex 18.1.a–d.

A higher body weight (trial 1) and/or a better feed to gain ratio (trials 1 and 3) were found in the birds receiving the recommended dose compared to the control in two trials. However, both trials showed limitations. In trial 1, the enzyme activity in feed was not confirmed. In trial 3, although the differences were reported as significant the statistical test to compare group means used does not correct for multiple comparisons (three groups). An adequate statistical analysis was not provided.

[30] Technical dossier/Section IV/Annexes 3.1.1.a–b.
[31] Technical dossier/Section III/Annexes 3.1.1.e–h.

Table 2: Effects of Bergazym® P100 on the performance of chickens for fattening

Trial	Treatments	Feed intake (g)(1)	Final body weight (g)	Feed to gain ratio	Mortality and culling (%)
1	0	5,825	2,857(b)	2.04(a)	4.5
	1,500	6,045	3,172(a)	1.91(b)	4.0
	3,000	6,020	3,125(a)	1.93(a),(b)	4.0
2	0	93.4	2,021	1.66	2.0
	1,500	93.8	2,052	1.63	0
3	0	97.8(a)	2,053(b)	1.70(a)	4.2
	1,500	96.6(a),(b)	2,106(a),(b)	1.64(b)	4.2
	300,000	93.6(b)	2,147(a)	1.56(c)	2.1

(a),(b): Within a column and within a trial, values with a different superscript are significantly different ($p < 0.05$).
(1): In trial 1, values are total feed intake and in trials 2 and 3, values are daily feed intake.

3.3.1.1. Conclusions in chickens for fattening

The FEEDAP Panel considers that there is insufficient evidence to conclude on the efficacy of Bergazym® P100 in chickens for fattening.

3.3.2. Efficacy in weaned piglets

Two short-term trials and three long-term trials in weaned piglets were submitted to support the efficacy of the additive.

3.3.2.1. Short-term trials

Two balance trials were considered which followed the same design and methodologies.[32,33]

Each balance study was performed with eight castrated male weaned piglets (hybrid, approx. 38 days old and initial body weight 16.9 kg in study 1 and 16.3 kg in study 2) housed in individual digestibility cages and allocated to two dietary treatments (four piglets per treatment). Each balance study was arranged in a 2 × 4 Latin square design: two phases (rotation) of 7 days each with 4 days of total collection of faeces and urine (separated) and an adaption period of 7 days. The basal diets, based on wheat, barley and soya bean meal, were either not supplemented (control) or supplemented with Bergazym® P100 to provide 1,500 EPU/kg feed. The enzyme activity was confirmed in the two studies. The diets were fed restrictively at 8% of piglets metabolic weight (body weight$^{0.75}$).

Body weight of the piglets was recorded at start, days 7, 14 and 21 and feed intake daily during collection period in both phases. Faeces and urine were totally collected from days 4 to 7 in both phases. Diets and faeces were analysed for dry matter, crude protein, crude fat and gross energy, and the apparent faecal digestibilities were calculated. Urine was analysed for nitrogen content and retention was calculated. The metabolisable energy content of the diets was calculated using the data on the apparent faecal digestibility and the energy excreted through urine (in the form of nitrogen). An ANOVA was performed with the data. Significance level was set at $p < 0.05$.

The metabolisable energy content of the diets for the first experiment was 15.0 and 15.4 MJ/kg feed for the control and the recommended dose group, respectively and the values were not significantly different. The corresponding figures for the second balance study were 14.1 and 14.4 MJ/kg feed, being the value in the recommended dose group significantly higher than in the control. Therefore, in one balance study, a higher metabolisable energy content of the diet was obtained in piglets fed the recommended dose.

3.3.2.2. Long-term trials

Three long-term trials were submitted. One of these trials, the first tolerance trial[23] (see Section 3.2.1.2), could not be considered further due to the extensive treatment with antibiotics that was administered to the piglets.

The first of the trials considered was the second tolerance trial described in Section 3.2.1.2.[24] In the second study considered,[24] a total of 144 weaned male and female piglets (hybrid (Topigs 20 × Tybor G), approx. 25 days old and initial body weight of 6.5 kg) were distributed to pens in

[32] Technical dossier/Section IV/Annex 4.2.2.1.a–i/Supplementary information November 2016/Annex 21.1.a–d.
[33] Technical dossier/Section IV/Annex 4.2.2.2.a–i/Supplementary information November 2016/Annex 21.2.a–d.

groups of six piglets each and allocated to two dietary treatments (representing 12 replicate pens per treatment). Basal diets (pre-starter and starter) based on wheat, barley, rye and soya bean were either not supplemented (control) or supplemented with Bergazym® P100 to provide 1,500 EPU/kg feed. Enzyme activity was confirmed by analysis. General health status and mortality were monitored daily. Feed and water were available *ad libitum* over an experimental period of 42 days. Body weight and feed intake were measured at days 1, 14 and 42, and feed to gain ratio calculated. An ANOVA was performed with the data. Significance level was set at $p < 0.05$.

The results of the two studies are presented in Table 3. No significant differences between the treatments were identified in any of the parameters evaluated.

Table 3: Effects of Bergazym® P100 on the performance of weaned piglets

Trial	Treatments	Feed intake (g/day)	Body weight (kg)		Feed to gain ratio	Mortality and culling (n)
			Initial	Final		
1	0	425	5.0	17.4	1.44	0
	1,500	423	5.0	17.5	1.42	6
	150,000	422	5.0	17.6	1.41	2
2	0	611	6.5	24.4	1.44	1
	1,500	617	6.5	24.6	1.43	0

3.3.2.3. Conclusions in weaned piglets

A higher metabolisable energy content of the diet was found in the piglets receiving the additive at the recommended dose in one trial. No other significant effects were found in the other three trials considered in the evaluation. Therefore, the FEEDAP Panel cannot conclude on the efficacy of Bergazym® P100 in weaned piglets.

3.3.3. Pigs for fattening

Two short-term trials and one long-term trial conducted in the same trial site were evaluated.

3.3.3.1. Short-term trials

Two balance trials were considered which followed the same design and methodologies.[34]

Each balance study was performed with eight female pigs for fattening (Italian Duroc) housed in individual digestibility cages and allocated to two dietary treatments (four pigs per treatment). The mean body weight of the pigs involved in the two studies was 35.7 kg (study 1) and 83.8 kg (study 2), respectively. Each balance study was arranged in a 2×4 Latin square design: two phases (rotation) of 7 days each with 4 days of total collection of faeces and urine (separated) and an adaption period of 7 days. The basal diets based on wheat, sorghum and soya bean meal, were either not supplemented (control) or supplemented with Bergazym® P100 to provide 1,500 EPU/kg feed. The enzyme activity was confirmed by analysis in the two studies. The diets were fed restrictively at 8% of the pigs' metabolic body weight (body weight$^{0.75}$).

Body weight of the pigs was recorded at start, days 7, 14 and 21 and feed intake daily during collection period in both phases. Faeces and urine were totally collected from days 4 to 7 in both phases. Diets and faeces were analysed for dry matter, crude protein, crude fat and gross energy, and the apparent faecal digestibilities were calculated. Urine was analysed for nitrogen content and retention was calculated. The metabolisable energy content of the diets was calculated using the data on the apparent faecal digestibility and the energy excreted through urine. An ANOVA was performed with the data. Significance level was set at $p < 0.05$.

The metabolisable energy content of the diets was 15.1 and 15.4 MJ/kg feed for the control and the recommended dose group in study 1 and 14.1 and 14.6 MJ/kg feed in study 2. The values obtained in the two studies for the pigs receiving the additive at the recommended dose were significantly higher compared to those found in the control diet.

[34] Technical dossier/Section IV/Annex 4.2.3.1.a-i. and Supplementary information November 2016/Annex 25.a-d.

3.3.3.2. Long-term trial

A total of 144 female and castrated male pigs (Italian Duroc, approx. 63 days old and initial body weight of 22.6 kg) were distributed to pens in groups of six pigs each (gender separated) and allocated to two dietary treatments (representing 12 replicate pens per treatment).[35] Basal diets (grower and finisher) based on wheat, sorghum and soya bean meal were either not supplemented (control) or supplemented with Bergazym® P100 to provide xylanase at 1,500 EPU/kg feed. The enzyme activity was confirmed by analysis. Health status and mortality were monitored daily. Feed and water were available *ad libitum* over an experimental period of 112 days. Body weight and feed intake were recorded at days 1, 56 and 112, and feed to gain ratio calculated. An ANOVA was performed with the data considering the pen as the experimental unit. Significance level was set at $p < 0.05$.

No pig died during the experiment. Mean daily feed intake was 2,030 and 2,010 g for control and recommended dose, respectively. The corresponding values for final body weight were 92.1 and 93.8 kg and for feed to gain ratio 3.27 and 3.18. Final body weight and feed to gain ratio were significantly higher in the pigs fed the recommended dose compared to control.

The FEEDAP Panel considers that in order to reflect different production conditions within the European Union, the trials should be carried out in at least two different locations. It is noted that the three studies in pigs for fattening were conducted in the same trial site. The Panel considers that the production conditions would have little impact on the parameters measured in short-term trials, and therefore this limitation is of little consequence for this assessment.

3.3.3.3. Conclusions for pigs for fattening

The additive has the potential to be efficacious in pigs for fattening at the recommended dose.

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[36] and Good Manufacturing Practice.

4. Conclusions

The additive is safe for chickens for fattening, weaned piglets and pigs for fattening at the recommended dose (1,500 EPU/kg feed).

The use of Bergazym® P100 as a feed additive does not give rise to concerns for consumers of animal products.

The additive is not irritant to skin or eyes but is considered a potential skin and respiratory sensitiser.

The use of Bergazym® P100 poses no risks to the environment.

The Panel cannot conclude on the efficacy of Bergazym® P100 for chickens for fattening and weaned piglets. The Panel concludes that Bergazym® P100 has the potential to be efficacious as a zootechnical additive in pigs for fattening at the recommended dose.

Documentation provided to EFSA

1) Bergazym® P100. August 2014. Submitted by Berg + Schmidt GmbH Co. KG.
2) Bergazym® P100. Supplementary information. November 2016. Submitted by Berg + Schmidt GmbH Co. KG.
3) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Bergazym® P100.
4) Comments from Member States.

References

EFSA (European Food Safety Authority), 2008a. Technical Guidance: Microbial Studies. EFSA Journal 2008;6 (10):836, 3 pp. doi:10.2903/j.efsa.2008.836

[35] Technical dossier/Section IV/Annex 4.3.3.1.a-i.

[36] 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.

EFSA (European Food Safety Authority), 2008b, revised in 2009. Technical Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for assessing the safety of feed additives for the environment. EFSA Journal 2008;6(9):803, 5 pp. doi:10.2903/j.efsa.2008.803

EFSA (European Food Safety Authority), 2008c. Technical Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for assessing the safety of feed additives for the environment. EFSA Journal 2008;6(10):842, 28 pp. doi:10.2903/j.efsa.2008.842

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Technical Guidance: Tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. doi:10.2903/j.efsa.2011.2175

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. doi:10.2903/j.efsa.2012.2536

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance for establishing the safety of additives for the consumer. EFSA Journal 2012;10(1):2537, 12 pp. doi:10.2903/j.efsa.2012.2537

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. doi:10.2903/j.efsa.2012.2539

Abbreviations

ANOVA	analysis of variance
CFU	colony-forming unit
CV	coefficient of variation
EC	European Commission
EURL	European Union Reference Laboratory
LOD	limit of detection
PCDD	polychlorinated dibenzo-*p*-dioxins
PCDF	polychlorinated dibenzofurans

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Bergazym® P100

In the current application authorisation is sought for *Bergazym P100* under article 4(1) under the category/functional 4(a) "zootechnical additives'/'digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for chickens for fattening, weaned pigs and pigs for fattening.

According to the Applicant, *endo-1,4-β-xylanase* produced by *Trichoderma reesei* (MUCL 49755) is the active substance of *Bergazym P100.* The Applicant expresses the *xylanase* enzymatic activity in endopentosanase units (EPU), defined as "the amount of enzyme which releases 0.0083 μmol of reducing sugars (xylose equivalent) per minute from oat spelt xylan at pH 4.7 and 50°C".

The *feed additive* is intended to be included through *premixtures* or directly in *feedingstuffs* to obtain a minimum activity of 1,500 EPU/kg in *feedingstuffs* for all the target species.

For the quantification of *xylanase* activity in the *feed additive, premixtures* and *feedingstuffs* the Applicant submitted a single-laboratory validated and further verified method based on the quantification of water soluble dyed fragments produced by the action of *xylanase* on a commercially available azurine cross-linked arabinoxylan substrate. External calibration is conducted using a reference standard with a known enzyme activity expressed in EPU. Based on the satisfactory performance characteristics the EURL recommends for official control the proposed single-laboratory validated and further verified colorimetric method for the quantification of the *xylanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*.