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Safety and efficacy of Belfeed B MP/ML (endo-1,4-beta-xylanase) as feed additive for poultry, piglets (weaned) and pigs for fattening

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

Belfeed B is a feed additive with endo-1,4-beta-xylanase as the main enzymatic activity, that is available in solid (MP) and liquid forms (ML). The production strain for Belfeed is a genetically modified strain of *Bacillus subtilis*. Belfeed B does not give rise to safety concerns with regard to the genetic modification of the production strain. Neither the production strain nor its recombinant DNA was detected in the final product obtained from the production strain. 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, laying hens and weaned piglets at the dose of 10 IU/kg feed and this conclusion was extended to chickens reared for laying, turkeys reared for breeding, breeding hens and pigs for fattening and extrapolated to minor poultry species at the same dose. The production strain belongs to a species considered to qualify for the qualified presumption of safety (QPS) approach to safety assessment provided that the qualifications are met. The identity of the strain was unambiguously established, the genetic modification raised no concerns and the relevant qualifications were met. Therefore, the FEEDAP Panel considered that the use of Belfeed B as a feed additive raises no concerns for consumers. Data from toxicological studies supported this conclusion. The additive is not considered to be toxic by inhalation or irritant for skin or eye, but it is considered a potential respiratory sensitiser. The Panel could not conclude on its skin sensitisation potential. The use of Belfeed B as a feed additive poses no risks to the environment. The Panel concluded that the additive has the potential to be efficacious at the dose of 10 IU/kg feed in the target species but owing to the lack of sufficient data the Panel cannot conclude on the efficacy of the product in turkeys for fattening.

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

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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 Belfeed B MP/ML as a feed additive for poultry species, piglets and pigs for fattening.

Belfeed B 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 for Belfeed B is a genetically modified strain of *Bacillus subtilis*. Belfeed B does not give rise to safety concerns with regard to the genetic modification of the production strain. Neither the production strain nor its recombinant DNA was detected in the final product obtained from the production strain.

Based on the tolerance trials evaluated the Panel concluded that the additive is safe for chickens and turkeys for fattening, laying hens and weaned piglets at the dose of 10 IU/kg feed. The Panel considered that the conclusions can be extended to chickens reared for laying, turkeys reared for breeding, breeding hens and pigs for fattening. Also and based on the wide margin of safety shown (100-fold) in major poultry species the Panel considers that the conclusions can be extrapolated to minor poultry species at the same dose.

The production strain belongs to a species considered to qualify for the qualified presumption of safety (QPS) approach to safety assessment provided that the qualifications are met. The identity of the strain was unambiguously established, the genetic modification raised no concerns and the relevant qualifications were met. Therefore, the FEEDAP Panel considered that the use of Belfeed B as a feed additive raises no concerns for consumers. Data from toxicological studies supported this conclusion.

The additive is not considered to be toxic by inhalation or irritant for skin or eye, but the Panel could not conclude on its skin sensitisation potential. Owing to the proteinaceous nature of the active substance, the additive is considered a potential respiratory sensitiser.

The use of Belfeed B as a feed additive poses no risks to the environment.

Based on the results obtained in the efficacy studies, the Panel concluded that the additive has the potential to be efficacious at the dose of 10 IU/kg feed in chickens for fattening, laying hens, weaned piglets and pigs for fattening. The Panel considers that this conclusion can be extended to chickens reared for laying and to breeding hens and can also be extrapolated to minor poultry species for fattening and or laying/breeding. Owing to the lack of sufficient data, the Panel cannot conclude on the efficacy of the product in turkeys for fattening.

Table of contents

Abstract 1
Summary 3
1. Introduction 5
1.1. Background and Terms of Reference 5
1.2. Additional information 5
2. Data and methodologies 6
2.1. Data 6
2.2. Methodologies 6
3. Assessment 6
3.1. Characterisation 6
3.1.1. Characterisation of the active substance 6
3.1.1.1. Information relating to the genetically modified microorganism 6
3.1.2. Manufacturing process 7
3.1.3. Characterisation of the additive 7
3.1.4. Shelf life and stability 8
3.1.4.1. Shelf life 8
3.1.4.2. Stability and homogeneity of the additive in premixtures 8
3.1.4.3. Stability and homogeneity of the additive in feed 8
3.1.5. Conditions of use 9
3.2. Safety 9
3.2.1. Safety of the genetic modification and of the strain 9
3.2.2. Safety for the target species 9
3.2.2.1. Safety for chickens for fattening 9
3.2.2.2. Safety for turkeys for fattening 10
3.2.2.3. Safety for laying hens 10
3.2.2.4. Safety for minor poultry species 10
3.2.2.5. Safety for piglets 11
3.2.2.6. Safety for pigs for fattening 11
3.2.2.7. Conclusions on safety for the target species 11
3.2.3. Safety for the consumer 11
3.2.4. Safety for the user 12
3.2.4.1. Effects on the respiratory system 12
3.2.4.2. Effects on the skin and eyes 12
3.2.4.3. Conclusions on safety for the user 12
3.2.5. Safety for the environment 12
3.3. Efficacy 13
3.3.1. Efficacy for chickens for fattening 13
3.3.2. Efficacy for turkeys 14
3.3.3. Efficacy for laying hens 15
3.3.4. Efficacy for ducks and other minor poultry species 16
3.3.5. Efficacy for weaned piglets 16
3.3.6. Efficacy for pigs for fattening 17
3.3.6.1. Conclusions on efficacy for the target species 19
3.4. Post-market monitoring 19
4. Conclusions 19
Documentation provided to EFSA 20
References 20
Abbreviations 21
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Belfeed B MP and Belfeed B ML 22

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. Article 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States. Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest 1 year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of 7 years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the company Beldem, a division of Puratos NV[2] for authorisation/re-evaluation of the product Belfeed B MP/ML, endo-1,4-beta-xylanase, when used as a feed additive for chickens for fattening, chickens reared for laying, laying hens, turkeys for fattening, turkeys for breeding purposes, turkeys reared for breeding, ducks, piglets (weaned), pigs for fattening and minor poultry species for fattening and laying (category: zootechnical additive; 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), Article 13(3) (modification of the authorisation of a feed additive) and under Article 10(2) (re-evaluation of an authorised 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 3 August 2011.

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

1.2. Additional information

The additive is a preparation of endo-1,4-beta xylanase produced by a genetically modified strain of *Bacillus subtilis* (LMG-S 15136). The additive, is authorised in the European Union, in the form of Belfeed B1100 MP/ML, as feed additive for chickens for fattening,[3] piglets,[4] ducks,[5] pigs and turkeys for fattening[6] and laying hens.[7] The currently authorised formulations, solid and liquid, have an enzyme activity of 100 IU[8]/g or mL.

The Scientific Committee on Animal Nutrition issued an opinion on the safety of the solid form of the additive for chickens for fattening and piglets (European Commission, 2002a) and for pigs for fattening (European Commission, 2002b), another one on the safety of the liquid form for chickens for fattening (European Commission, 2002c), and one on the safety and on the safety of the solid and

[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] Beldem, a division of Puratos NV, Rue Bourrie 12, 5300 Andenne, Belgium.
[3] Commission Regulation (EC) No 1259/2004 of 8 July 2004 concerning the permanent authorisation of certain additives already authorised in feedingstuffs. OJ L 239, 9.7.2004, p. 8.
[4] Commission Regulation (EC) No 1206/2005 of 27 July 2005 concerning the permanent authorisation of certain additives in feedingstuffs. OJ L 197, 28.7.2005, p. 12.
[5] Commission Regulation (EC) No 242/2007 of 6 March 2007 concerning the authorisation of endo-1,4-beta-xylanase EC 3.2.1.8 (Belfeed B1100MP and Belfeed B1100ML) as a feed additive. OJ L 73, 13.3.2007, p. 1.
[6] Commission Regulation (EC) No 516/2007 of May 2007 concerning the permanent authorisation of an additive in feedingstuffs. OJ L 122, 11.5.2007, p. 22.
[7] Commission Regulation (EC) No 322/2009 of 20 April 2009 concerning the permanent authorisations of certain additives in feedingstuffs. OJ L 101, 21.4.2009, p. 9.
[8] One International Unit (IU) is defined as the amount of enzyme which liberates 1 μmol of reducing sugars (xylose equivalents) from birchwood xylan per minute at pH 4.5 and 30°C.

liquid forms for turkeys for fattening (European Commission, 2002d). EFSA issued an opinion on the safety of the product for laying hens (EFSA, 2004) and on the safety and efficacy for ducks (EFSA, 2006).

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[9] in support of the authorisation request for the use of product substance 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 and the applicable EFSA guidance documents.

EFSA has verified the 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.[10]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Belfeed B is in line with the principles laid down in Regulation (EC) No 429/2008[11] 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,b), 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 risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011).

3. Assessment

The additive Belfeed B is intended for use in chickens for fattening, chickens reared for laying, laying hens, turkeys for fattening, turkeys for breeding purposes, turkeys reared for breeding, ducks, piglets (weaned), pigs for fattening and minor poultry species for fattening and laying as a zootechnical additive (digestibility enhancers). The current assessment deals with the re-evaluation of the additive, however, a change in the final formulations of the additive has also been proposed. The applicant is requesting also to extend the authorisation to poultry species/categories other than those in which is already authorised.

3.1. Characterisation[12]

3.1.1. Characterisation of the active substance

The main activity contained in the additive is endo-1,4-beta-xylanase (EC 3.2.1.8; xylanase). This xylanase is produced by a genetically modified strain of *Bacillus subtilis*. Other side enzyme activities may be present in the final additive, beta-glucanase and alpha-amylase.

3.1.1.1. Information relating to the genetically modified microorganism

The production strain is *B. subtilis*, deposited at the LMG culture collection (Belgian Co-ordinated Collections of Microorganisms, University of Ghent) with deposition number LMG S-15136. The strain was identified by phylogenetic analysis of the gene sequence of the 16S ribosomal RNA,[13] cell morphology, biochemical traits and fatty acid profile.[14]

[9] FEED dossier reference: FAD-2010-0285.
[10] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2010-0285-belfeed.pdf
[11] 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.
[12] This section has been amended following the confidentiality claims made by the applicant.
[13] Technical dossier/Supplementary information September 2014.
[14] Technical dossier/Section II/Annex II.09.

The parental strain is *Bacillus subtilis* Marburg 168. *B. subtilis* has been recommended for qualified presumption of safety (QPS) with the qualification that the absence of toxigenic potential has to be verified for the strain used (EFSA BIOHAZ Panel, 2013). The genome of strain *B. subtilis* Marburg 168 has been fully sequenced and is publicly available. Bioinformatic analyses searching for homology with known toxins and surfactins, PCR detection of non-ribosomal peptide synthase genes to identify surfactin-positive strains, and tests for haemolysis and cytotoxicity tests with Vero cells yielded negative results for the parental strain *B. subtilis* Marburg 168, indicating that this strain is not toxigenic.[15] The technical dossier contains detailed and sufficient information on the recipient microorganism, including aspects on the safety of the strain lineage, the origin and function of the different genetic elements introduced in the production strain, the genetic modification process and the genetic and phenotypic traits introduced.[16–18]

3.1.2. Manufacturing process

Belfeed B is produced by submerged, pure culture fermentation of the production strain. The enzyme is secreted into the culture broth. The substrate (sugar, proteins, salt) is mixed, sterilised and fermented by *Bacillus subtilis* in a closed system. The fermentation product, containing xylanase (and side activities beta-glucanase and alpha-amylase) is separated from the biomass by micro-filtration. The enzymatic filtrate is concentrated. As a result of a modification of the manufacturing, the resulting product has a higher enzyme activity than in the past which permits to prepare formulations with higher enzyme activity. The enzyme concentrate is filtered and spray-dried on a carrier (food grade wheat flour) or mixed with glycerol to produce the two preparations of the additive, solid and liquid, respectively. No antimicrobials are used in the manufacturing of the product.

3.1.3. Characterisation of the additive

The additive is available in solid and liquid forms.

The solid formulation, Belfeed B MP, ensures a minimum of 400 IU^8/g. The study of the batch-to-batch variation in six batches showed a mean value of 420 IU/g ranging from 412 to 434 IU/g (coefficient of variation (CV) 1.9%).[19] This formulation contains the liquid enzyme concentrate (including the elution acetate buffer) spray-dried on food grade wheat flour (minimum of 90%). The particle size of three batches of the additive was measured by laser diffraction and showed a particle size of 107 μm (median value) with less than 25% (v/v) of the particles with a diameter of 50 μm and less than 4% (v/v) of the particles with a diameter of 10 μm.[20] The dusting potential was measured in one batch using the Stauber–Heubach method (triplicate), the dust amounted 0.187 g/m^3.[21] The bulk density is 660 kg/m^3.

The liquid formulation, Belfeed B ML, also ensures a minimum of 400 IU/g. The study of the batch-to-batch variation in five batches showed a mean value of 428 IU/g ranging from 416 to 441 IU/g (CV 2.5%).[22] This formulation contains the liquid enzyme concentrate (including the elution acetate buffer) and glycerol (maximum 30%). It has a density similar to 1,050–1,100 kg/m^3,[23] a viscosity of 2.65–2.70 centipoises (at 20°C) and a pH of 4.19–4.22.[24]

Three batches of the enzyme concentrate were analysed for chemical and microbial contamination.[25] Microbial contamination included the study of *Salmonella* spp. (absent/25 g), *Bacillus cereus* (< 100 colony forming units (CFU)/g), coagulase-positive *Staphylococcus* (< 10 CFU/g), coliforms (30°C, < 10 CFU/g), β-glucuronidase positive *E. coli* (< 10 CFU/g), yeasts (100 CFU/g), filamentous fungi (100 CFU/g), enterococci (< 100 CFU/g), *Clostridium perfringens* (10 CFU/g) and sulphite-reducing anaerobic bacteria 37°C (< 10 CFU/g). The chemical contamination included the study of arsenic (< 0.05 mg/kg), cadmium (< 0.01 mg/kg), mercury (< 0.01 mg/kg), lead (0.02 mg/kg) and mycotoxin deoxynivalenol (< 50 μg/kg), aflatoxins B1, B2, G1, G2 (< 1 μg/kg), T2 toxin (< 20 μg/kg), HT2 toxin

[15] Technical dossier/Section III/Annex III.13.
[16] Technical dossier/Supplementary information June 2013 and November 2014.
[17] Technical dossier/Confidential parts/Annex III.13 conf.
[18] Technical dossier/Supplementary information March 2014, September 2014 and November 2014.
[19] Technical dossier/Supplementary information June 2013/Annex Q05-1.
[20] Technical dossier/Supplementary information June 2013/Annex Q07-1.
[21] Technical dossier/Supplementary information March 2014/Annex Q(ii)_1 and 2.
[22] Technical dossier/Supplementary information June 2013/Annex Q-05-1.
[23] Technical dossier/Section II.
[24] Technical dossier/Supplementary information March 2014/Annex Q(iii)_1 to 3.
[25] Technical dossier/Supplementary information June 2013/Annex Q06-1.

(< 20 μg/kg), fumonisine B1 and B2 (< 50 μg/kg), nivalenol (< 200 μg/kg), α and β-zearalenol (< 20 μg/kg), ochratoxin A (< 0.50 g/kg) and cytochalasin E (< 2 μg/kg). Antimicrobial activity was found to be absent when analysed following the method specified by the Joint FAO/WHO Expert Committee on Food Additives (FAO JECFA, 2006).[26] The production strain was not detected in a test volume of 1 mL of three batches of the liquid concentrate before formulation, each sample tested in triplicate.[27] No recombinant DNA from the production strain *B. subtilis* was detected in four batches of concentrate before formulation, each tested in triplicate by PCR.[27]

3.1.4. Shelf life and stability

3.1.4.1. Shelf life

Three batches of the solid and the liquid formulations were stored in closed packages at 25°C for 13 or 12 months, respectively, or at 40°C for 16 or 12 weeks, respectively.[28] The samples of the liquid formulation were also kept at 4°C for 12 months. The initial enzyme activity ranged 416–434 IU/g for the solid and 416–441 IU/g for the liquid. After 12 months of storage at 25°C, the recoveries of the two formulations showed no modifications of the initial enzyme activity. After 16 weeks at 40°C, the solid formulation showed recoveries of 94%, the recoveries of the liquid formulation were below 60% after 3 weeks. After 12 months, no modifications on the enzyme activity were found in the liquid formulation kept at 4°C.

3.1.4.2. Stability and homogeneity of the additive in premixtures

Three batches of the solid and liquid formulations were added to three different complete premixtures: one for chickens for fattening, one for laying hens and one for pigs (containing choline chloride).[29] Supplementation was at 1,000 IU/kg premixture for the pig and chickens and at 500 IU/kg premixture for the layers. The samples were stored in sealed plastic bags at room temperature (not exceeding 25°C) for 24 weeks. Recoveries were similar to 100% regardless of the premixture and additive form considered.

Six subsamples of premixtures supplemented with the solid or with the liquid formulations were analysed to check the capacity of the additive to homogeneously distribute. The average enzyme activity for the solid form was 1,029 IU/kg premixture and for the liquid it was 1,027 IU/kg. The CV of the samples analysed for the solid was 3% and for the liquid it was of 4%.

3.1.4.3. Stability and homogeneity of the additive in feed

Three batches of the solid formulation were added to two mash diets, one for chickens for fattening, and one for pigs, based on cereals and soya bean meal.[30] Supplementation was at the recommended level and the recoveries were measured after 12 weeks. The samples were kept in sealed plastic bags at room temperature (not exceeding 25°C). Recoveries were higher than 90%. A further batch was added to a mash feed for piglets at three dosages (10, 25 and 50 IU/kg feed) and kept at room temperature for 24 weeks. No losses on the enzyme activity were recorded.[23]

Two batches of a diluted version of the solid formulation (currently authorised) were added to mash feed for chickens for fattening (based on maize and soya bean meal) and then pelleted at 65°C.[31] Samples were stored in sealed plastic bags at room temperature (not exceeding 25°C). Measurements of xylanase were performed after pelleting and after 12 weeks. Recoveries were higher than 96% in all cases. The effect of the heat process on the enzyme activity was studied in two more batches of a diluted version of the solid formulation.[23] One batch was added to feed at 2,000 IU/kg and subject to pelleting at 65, 75 or 85°C (or not pelleted). Recoveries were 86%, 67% and 89% compared to the non-pelleted diet. Another batch was added to feed at 10 IU/kg and pelleted at 75°C, and the enzyme activity was followed for 24 weeks; no details were available on the storage conditions. The losses after pelleting were similar to 25% and thereafter the enzyme activity remained stable for the 24 weeks.

Three batches of the liquid formulation were added to a pelleted diet for chickens for fattening based on maize and soya bean meal.[32] Supplementation was at the recommended level and the

[26] Technical dossier/Supplementary information September 2014/Annex 1.
[27] Technical dossier/Supplementary information June 2013.
[28] Technical dossier/Supplementary information June 2013/Annex Q08-1 and Supplementary information March 2014.
[29] Technical dossier/Supplementary information June 2013/Annex Q11-1 and Supplementary information March 2014.
[30] Technical dossier/Supplementary information June 2013/Annex Q13-1 and Supplementary information March 2014.
[31] Technical dossier/Supplementary information June 2013/Annex Q13-2 and Supplementary information March 2014.
[32] Technical dossier/Supplementary information June 2013/Annex Q13-3 and Supplementary information March 2014.

recoveries were measured after 12 weeks. The samples were stored in sealed plastic bags at room temperature (not exceeding 25°C). Recoveries after 12 weeks were higher than 92%. A further batch was added to a mash feed based on cereals and cereal by-products at 10 IU/kg feed and stored at room temperature for 16 weeks. No losses on the enzyme activity were found.[23]

The capacity of the solid and liquid formulations to homogeneously distribute was studied when added to a feed for pigs at the recommended dose in mash and/or pelleted feed.[33] Three batches of each formulation were studied and 10 subsamples of the feeds were analysed. The CV of the enzyme activity in feed supplemented with the solid formulation ranged from 4.1% to 7.9% for the mash feed and from 4.6% to 8.7% in the pelleted feed and the CV for the pelleted feeds supplemented with the liquid formulation ranged from 4.4% to 4.8%.

3.1.5. Conditions of use

Belfeed B MP/ML is intended to be used at a dose of 10 IU/kg feed in complete feed for chickens for fattening or reared for laying, turkeys, laying hens, ducks, other minor poultry species for fattening and laying, weaned piglets and pigs for fattening. The liquid formulation is to be added to the feed mainly after pelleting.

3.2. Safety

3.2.1. Safety of the genetic modification and of the strain[34]

The parental organism, *B. subtilis* Marburg 168, is considered to be safe. The introduced trait is well known and does not raise any safety concern. Analyses revealed that neither the production strain nor the recombinant DNA was present in the final product. The final product does not raise any safety concern with regard to the genetic modification of the production strain.

3.2.2. Safety for the target species

The test materials used in the tolerance studies were not the products described in the characterisation section, but the currently authorised formulation of the additive (four times diluted). The Panel considers that these test materials are adequate and will be referred in the studies below as Belfeed B xylanase (the form is indicated).

3.2.2.1. Safety for chickens for fattening

A total of nine hundred 1-day-old male chicks (Ross 308) were distributed in 30 pens of 30 birds each and allocated to three dietary treatments (10 replicates per treatment).[35] The basal diets (starter, grower and finisher) based on wheat, maize and soya bean meal were supplemented with Belfeed B xylanase (solid) at 0, 10 (1×) or 1,000 (100×) IU/kg feed. The enzyme activities were confirmed by analysis. The feed was offered to the birds *ad libitum* in mash form for 39 days (starter, 1–13 days; grower, 13–26 days; finisher, 26–39 days). Mortality and health status were monitored throughout the study. Feed consumption and body weight of the birds were measured when the diets were changed and at the end of the study. An analysis of variance (ANOVA) was done on the data, pen basis, means were compared using the Dunnett's test and differences were considered significant at a level of at least $p < 0.05$.

Mortality was low and amounted to 1.3% in all treatments. Mean daily feed intake of the birds was 104, 104 and 106 g for 0, 1× and 100×, respectively, and was not affected by the treatments. Final body weight was 2.51, 2.61 and 2.67 kg for 0, 1× and 100×, respectively, and the corresponding figures for the feed to gain ratio were 1.64, 1.59 and 1.57. The supplementation of the xylanase at either dose resulted in a statistically significant higher body weight and better feed to gain ratio as compared to the control diet. Feeding the birds with Belfeed B xylanase up to 100-fold the recommended dose did not have any negative effects on the performance parameters. Therefore, the FEEDAP Panel concludes that the product is safe for chickens for fattening at the recommended dose.

[33] Technical dossier/Supplementary information January 2016/Annex 1.
[34] This section has been amended following the confidentiality claims made by the applicant.
[35] Technical dossier/Supplementary information June 2013 Annex Q16-1 and Q16-2 and supplementary information March 2014/Annex Q(vi)_1/Supplementary information June 2016b.

3.2.2.2. Safety for turkeys for fattening

A total of four hundred and thirty-two 1-day-old male turkeys (Hybrid Grade makers) were distributed in 24 cages of 18 birds each (cages of 2.6 m^2) and allocated to three dietary treatments (eight replicates (cages) per treatment).[36] The basal diets (starter and grower) based on wheat, corn and soybean meal were supplemented with Belfeed B xylanase (solid) at 0, 10 (1×) or 1,000 (100×) IU/kg feed. This diet contained monensin. The enzyme activities were confirmed by analysis. Feed was offered to the birds *ad libitum* for 42 days. The feed was offered in crumble form during the starter phase (0–21 days) and granulated during the grower phase (21–42 days). Mortality and health status were monitored throughout the study. Feed consumption and body weight of the birds were measured on days 21 and 42 of the study by cage. Feed to gain ratio was calculated. An ANOVA was done on the performance data, cage basis and the initial body weight was considered as covariate in the model. The differences were considered significant at a level of at least $p < 0.05$.

No animals died; only two were culled (one from 0 and another one from 100×). Mean daily feed intake of the birds was 99, 100 and 97 g for 0, 1× and 100×, respectively, and was not affected by the treatments. Final body weight was 2.94, 2.96 and 2.95 kg for 0, 1× and 100×, respectively, and the corresponding figures for the feed to gain ratio were 1.44, 1.46 and 1.41. The supplementation of Belfeed B xylanase at 100× dose resulted in a statistically significant better feed to gain ratio as compared to the control diet. Feeding the birds with the xylanase up to 100-fold the recommended dose did not have any negative effects on the performance parameters. Therefore, the FEEDAP Panel concludes that the product is safe for turkeys for fattening at the recommended dose.

3.2.2.3. Safety for laying hens

A total of two hundred and eighty-eight 16-week-old hens (Hy-Line Brown) were distributed in 36 enriched cages of eight hens each. From 16 to 17 weeks of age, the animals were fed a pre-laying commercial diet without enzyme additive. At 23 weeks, they were allocated to three dietary treatments (12 replicates (cages) per treatment).[37] A basal diet based on wheat, rye and soya bean meal was supplemented with Belfeed B xylanase (solid) at 0, 10 (1×) or 1,000 (100×) IU/kg feed. The enzyme activities were confirmed by analysis. The feed was offered in mash form and *ad libitum* for 56 days. 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 at day 28 and day 56 per cage. 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 $p < 0.05$.

No mortality was reported. Mean daily feed intake of the hens was 114, 110, 111 g for 0, 1× and 100×, respectively, and was significantly lower in the diets supplemented with xylanase (1×) compared to the control. Laying rate was 95.8%, 95.4% and 94.7% for 0, 1× and 100×, respectively, and the corresponding figures for egg weight were 58.8, 59.1 and 58.1 g and the feed to egg mass ratio were 2.03, 1.95 and 2.02. No statistically significant differences were observed for these parameters in the 100× group compared to the control. The addition of xylanase at the recommended dose (1×) improved significantly the feed to egg mass ratio compared to the other treatments. Feeding the birds with xylanase up to 100-fold the recommended dose did not have any negative effects on the performance parameters. Therefore, the FEEDAP Panel concludes that the product is safe for laying hens at the recommended dose.

3.2.2.4. Safety for minor poultry species

The applicant submitted a trial with Muscovy ducks which had been previously evaluated by the FEEDAP Panel (EFSA, 2006). In that trial, three treatments were considered – a non-supplemented diet and two diets supplemented with the Belfeed B xylanase at 10 (1×) or 100 (10×) IU/kg feed. Mortality and health status were monitored. Performance of the birds was also measured. The results showed no adverse effects of the additive on the performance of the birds at the highest dose, but no data were available on the haematological/biochemical parameters in blood.

[36] Technical dossier/Supplementary information June 2013 Annex Q16-3 and Supplementary information March 2014/Annex Q(vi)_2.
[37] Technical dossier/Supplementary information June 2013 Annex Q16-4 and Q16-5 and Supplementary information March 2014/Annex Q(vi)_3 and Annex Q(vii)_1.

Based on the wide margin of safety shown, the conclusions from the major poultry species can be extrapolated to minor poultry species. The results obtained in the study in ducks are considered as supportive evidence on the safety of the product in minor poultry species for fattening.

3.2.2.5. Safety for piglets

A total of 144 male and female piglets (Pietrain × (Landrace × Large white), 21 days old) were distributed randomly in a total of 24 floor pens in groups of six piglets and allocated to three dietary treatments (eight replicates per treatment).[38] Upon arrival, the animals were administered an intramuscular dose of tulathromycin and from day 21 to day 26 of life, piglets were fed a post-weaning adaptation diet (medicated feed). The piglets received the experimental diets from day 26 of life (mean body weight 6.7 kg). Two basal diets (pre-starter and starter) based on wheat, milk whey, rye, barley and soya bean meal were supplemented with Belfeed B xylanase at 0, 10 (1×) or 1,000 IU/kg feed (100×). The enzyme activity was confirmed by analysis. The feed was offered in pelleted form and *ad libitum* for 42 days. Mortality and health status were monitored throughout the study. Body weight and feed intake were measured on days 14, 29 and 42 of study (body weight at the beginning as well). An ANOVA was performed on the data obtained. The model included the effect of the diet and the block (weight and location), and differences were considered significant at a level of at least $p < 0.05$.

Four pigs of the non-supplemented diet died, three in treatment 1× and two on treatment 100×. The mean daily feed intake was 569, 570 and 555 g for 0, 1× and 100×, respectively, and the corresponding figures for final body weight were 25.3, 25.5, 25.7 kg and for feed to gain were 1.30, 1.28 and 1.23. The 100× treatment showed a better feed to gain ratio compared to the other groups. Feeding the piglets with xylanase up to 100-fold the recommended dose did not have any negative effects on the performance parameters. Therefore, the FEEDAP Panel concludes that the additive is safe for weaned piglets at the recommended dose.

3.2.2.6. 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 piglets can be extended to pigs for fattening.

3.2.2.7. Conclusions on safety for the target species

The tolerance trials provided in chickens and turkeys for fattening, laying hens and weaned piglets showed that the animals can tolerate up to 100-fold the recommended dose of 10 IU xylanase/kg feed. Therefore, the FEEDAP Panel concludes that the additive is safe for these target species/categories at that dose.

The Panel considers that the conclusions reached for the chickens and turkeys for fattening can be extended to chickens reared for laying and turkeys reared for breeding, and the conclusions for laying hens can be extended to breeding hens at the same dose. Similarly, the conclusions for weaned piglets can be extended to pigs for fattening at the same dose.

Based on the wide margin of safety shown, the conclusions on the safety for the major poultry species can be extrapolated to ducks and other minor poultry species for fattening and for laying at the same dose.

3.2.3. Safety for the consumer

The toxicological studies are not required if the fermentation product is produced by a genetically modified microorganism that is considered by EFSA to qualify for the QPS approach to safety assessment and 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, the genetic modification raised no concerns and the relevant qualifications were met. Therefore, the FEEDAP Panel concludes that the use of Belfeed B as a feed additive raises no concerns for consumers.

The applicant provided various toxicity studies to support the safety of the product: two acute toxicity studies, four genotoxicity studies and one sub-chronic oral toxicity study. The two acute oral

[38] Technical dossier/Supplementary information June 2013 Annex Q16-6 and Supplementary information March 2014/Annex Q(vi)_2.

toxicity studies,[39,40] did not show any sign of acute toxicity. The two bacterial reverse mutation assays,[41,42] performed in *Salmonella* Typhimurium (strains TA 1535, TA 1537, TA 98 and TA 100) and in one case in *Escherichia coli* CM8991 (WP2uvrA/pKM101) showed no evidence of mutagenic activity. No evidence of clastogenic activity was found in an *in vitro* chromosomal aberration test in human lymphocytes[43] and no induction of micronuclei was reported in the *in vivo* mammalian micronucleus test.[44] Moreover, no treatment-related adverse effects were reported in the sub-chronic oral toxicity test performed in rats.[45] Although in most of the provided studies, with the exception of one of the genotoxicity studies,[44] the test item was not derived from the manufacturing process that is currently used to obtain the xylanase, the Panel considers these data supportive of the safety of the product.

3.2.4. Safety for the user

The studies under this section were performed with the fermentation product that was used in the past to obtain the additive, below referred as test item.[46] Considering the characteristics of the products obtained after the fermentation, the Panel considers that the results obtained in the below studies are relevant to the fermentation product that is currently used to formulate the additive.

3.2.4.1. Effects on the respiratory system

An acute inhalation toxicity study was performed on Sprague-Dawley rats exposed for 4 h to an aerosol containing a mean concentration of 6.08 mg test item/L, according to OECD Guideline 403.[47] No treatment-related signs were reported during the observation period or at the end of the study.

Owing to the proteinaceous nature of the active substance, the additive is considered as a potential respiratory sensitiser. The solid form has less than 4% of particles with a diameter of 10 μm and therefore there is a potential exposure by inhalation; however, the dusting potential is low.

3.2.4.2. Effects on the skin and eyes

An acute dermal irritation study was performed on three New Zealand White rabbits, according to OECD Guideline 404.[48] The test item was tested by a single exposure to 0.5 mL of the material for 4 h. Responses were recorded after 1, 24, 48 and 72 h and no dermal reaction was detected.

The potential of the test item to induce eye irritation was assessed in three New Zealand White rabbits according to the OECD Guideline 405.[49] Ocular reactions were assessed at 1, 24, 48 and 72 h after treatment. Very slight conjunctiva redness was observed after 1 and 24 h. The eyes were overtly normal at the 48-h examination. Therefore, under the conditions of this test, the test item was classified as non-irritant to the eye.

No data were provided on dermal sensitisation potential.

3.2.4.3. Conclusions on safety for the user

The studies under this section were performed with the fermentation product that was used in the past to formulate the product. The results are considered to be relevant to the fermentation product that is currently used to formulate the additive. The fermentation 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 skin 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

Neither the production strain nor its recombinant DNA was detected in the final product. The final product does not raise any environmental safety concern associated with the genetic modification of

[39] Technical dossier/Section III/Annex III.11.
[40] Technical dossier/Section III/Annex III.7.
[41] Technical dossier/Section III/Annex III.9.
[42] Technical dossier/Section III/Annex III.8.
[43] Technical dossier/Section III/Annex III.10.
[44] Technical dossier/Supplementary information June 2013/Annex Q17.1.
[45] Technical dossier/Section III/Annex III.12.
[46] Technical dossier/Supplementary information March 2014.
[47] Technical dossier/Section III/Annex III.14.
[48] Technical dossier/Section III/Annex III.15.
[49] Technical dossier/Section III/Annex III.16.

the production strain. 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

The test materials used in the efficacy studies were not the products described in the section on characterisation, but the currently authorised formulations of the additive, diluted four times. The Panel considers that these test materials are adequate and will be referred in the studies below as Belfeed B xylanase. Indications on the formulation, solid or liquid are also provided.

3.3.1. Efficacy for chickens for fattening

A total of 11 efficacy trials (including the tolerance trial) were evaluated. Eight trials were not considered further in the assessment due to different reasons: one trial due to the short duration and the type of parameters measured (28 days, performance), two more due to the high mortality (> 6%)[50] and the other five trials[51] could not be considered due to the fact that the raw data were not available anymore and the statistical analysis of the data could not be redone according to the current requirements. Therefore, the trials that were considered in the assessment were one short-term trial and two long-term trials.

In the short-term trial,[52] one hundred and forty-four 1-day-old male chickens (Cobb) were distributed to cages in groups of six, according to the body weight, and the cages were allocated to one of the four dietary treatments (representing six replicates per treatment). The study followed a 2 × 2 factorial design with two basal diets differing in the metabolisable energy (ME) content (gross energy content 18.40 or 19.57 MJ/kg feed dry matter basis) and two supplemented levels of Belfeed B xylanase in solid form (0 or 10.5 IU/kg feed, analysed doses 8.7 and 9.3 IU/kg feed for low energy and high energy content, respectively). The basal diets were based on wheat and soya bean meal and differed in the oil content. The animals were under study for 14 days and a balance took place for 3 days. Feed intake and body weight of the birds were measured during the study, and feed and excreta samples were analysed in order to study the energy content. An ANOVA was performed with the data in order to study the effect of the energy content and the effect of the enzyme and their interaction. The results showed that the addition of the enzyme at 10.5 IU/kg feed resulted in a significantly higher ME content of the diets (apparent ME nitrogen-corrected control: 13.93 Belfeed B xylanase: 14.19 MJ/kg dry matter feed) compared to control.

In the first long term trial, a total of five hundred and forty 1-day-old male and female chickens (Ross) were distributed (sex separated) to pens in groups of 30 birds and allocated to three dietary treatments, whereas at the start of the growing period, the animals were split into two pens (representing 12 replicates per treatment).[53] The basal diet based on wheat and soya bean meal was either not supplemented (control) or supplemented with Belfeed B xylanase in solid or liquid form at 10.5 IU/kg feed. The enzyme activities were confirmed by analysis (10.4 and 10.3 IU/kg feed). Feed was offered to the birds *ad libitum* for 42 days. Mortality and health status were monitored throughout the study. Feed consumption and body weight of the birds was measured on days 14 and 42 of the study. Feed to gain ratio was calculated. An ANOVA was done on the performance data, pen basis, considering the effect of the treatment, the sex and their interaction and mean values were compared using the Dunnett method.[54] The differences were considered significant at a level of at least $p < 0.05$. The results are presented in Table 1, trial 1. The results showed a significant effect of the enzyme, in either form, on the body weight and feed to gain ratio of the chickens.

The second long-term trial is the tolerance trial in chickens for fattening that has been presented in Section 3.2.2 and the results are shown in Table 1, trial 2. There was a significant effect of the additive on the final body weight and on the feed to gain ratio.

[50] Technical dossier/Section IV/Annex.IV.01.01, 01.04 and 01.06.
[51] Technical dossier/Section IV/Annex IV.01.02, 01.03, 01.07, 01.08 and 01.09.
[52] Technical dossier/Section IV/Annex IV.01.10.
[53] Technical dossier/Section IV/Annex IV.01.05/Supplementary information June 2016/Annex.
[54] Data on the daily feed intake were not evaluated using this methodology; means in the table were compared using LSMEANS.

Table 1: Effects of Belfeed B on the performance of chickens for fattening

Trial	Enzyme activity (IU/kg)	Daily feed intake (g)	Final body weight (g)	Feed to gain	Mortality and culling (%)
1	0	75[b]	1,875[b]	1.73[a]	2.1
	10.5 solid	78[a]	1,995[a]	1.69[b]	1.1
	10.5 liquid	78[a]	2,006[a]	1.68[b]	2.1
2	0	104	2,515[b]	1.64[a]	1.3
	10	104	2,608[a]	1.59[b]	1.3
	1,000	106	2,671[a]	1.57[b]	1.3

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

The addition of the enzyme to the feed resulted in an increased ME content in one trial and in a higher body weight and better feed to gain ratio in two trials. Therefore, the FEEDAP Panel concludes that Belfeed B xylanase is efficacious at the dose of 10 IU/kg feed in chickens for fattening. This conclusion can be extended to chickens reared for laying.

3.3.2. Efficacy for turkeys

A total of six trials were evaluated, one[55] was not further considered in the assessment due to the lack of a control (i.e. the non-supplemented diet had a different composition compared to the diets supplemented with the additive) and another was not further considered due to the high mortality/culling registered during the study (> 10%).[56]

The details of the design of the other four studies are provided in Table 2 and the results in Table 3. In all trials, 1-day-old birds were used, in trials 1 and 4, males were used and in trial 3, females were used. In trial 2, males and females were used and were kept sex separated. In the four studies the birds were treated either with a non-supplemented diet (control) or with a diet containing Belfeed B xylanase at 10 IU/kg feed (confirmed by analysis). Health and mortality were monitored throughout the study, and body weight and feed intake were recorded. Feed to gain ratio was calculated. The data were analysed with an ANOVA, pen basis.

Table 2: Trial design and dosages of the efficacy trials performed in turkeys for fattening

Trial	Total no. animals (animals/replicate) replicates/treatment	Breed Duration Sex	Diet composition	Enzyme activity (IU/kg feed)	
				Intended	Analysed
1[(a)]	486 (27) 6	BUT 9 16 w ♂	Wheat, soya bean meal	0 10.5 solid 10.5 liquid	0 8.7–9.4 8.8–9.4
2[(b)]	300♂/372♀ (25/31) 8	BUT 9 16/12 w ♂/♀	Wheat, soya bean meal	0 10.5 solid 10.5 liquid	0 8.5–9.5 7.9–9.1
3[(c)]	320 (40) 4	BUT 9 15 w ♀	Wheat, triticale, soya bean meal	0 10.5 solid	0 8.2–10.5
4[(d)]	264 (11) 12	BUT 9 15 w ♂	Wheat, soya bean meal	0 10.5 solid	0 7.5–10

(a): Technical dossier/Section IV/Annex 02.01/Supplementary information June 2013 and Supplementary information June 2016a/Annex 6.
(b): Technical dossier/Section IV/Annex 02.02 and supplementary information June 2013/Annex Q21-1.
(c): Technical dossier/Section IV/Annex 02.03/Supplementary information June 2013 and Supplementary information June 2016b/Annex 2.
(d): Technical dossier/Section IV/Annex 02.04 and supplementary information June 2013/Annex Q23-1.

[55] Technical dossier/Section IV/Annex 02.05 and supplementary information June 2013.
[56] Technical dossier/Supplementary information June 2013/Annex Q24-4.

Xylanase resulted in a significantly higher final body weight in trials 3 and 4 and in a better feed to gain ratio in trial 4. Positive and significant effects of the additive were seen in only two trials in turkeys; therefore, the Panel is not in a position to conclude on the efficacy of Belfeed B in turkeys for fattening.

Table 3: Effects of Belfeed B xylanase on the performance of turkeys for fattening

Trial	Treatments	Sex	Feed intake (kg)(1)	Final body weight (kg)	Feed to gain ratio	Mortality and culling (%)
1	0	Males	0.30	11.5	3.02	8.0
	10.5 solid		0.29	11.5	2.92	8.6
	10.5 liquid		0.29	11.6	2.84	8.0
2	0	Females	13.5	6.1	2.25	2.5
	10.5 solid		13.4	6.2	2.19	1.6
	10.5 liquid		13.6	6.3	2.20	1.7
	0	Males	30.8	12.8	2.45	1.1
	10.5 solid		30.8	12.9	2.41	6.8
	10.5 liquid		30.7	13.0	2.39	5.6
3	0	Females	0.15	8.1[b]	2.56	1.9
	10.5 solid		0.15	8.6[a]	2.40	1.9
4	0	Males	23.2	11.0[b]	2.13[a]	3.8
	10.5 solid		23.2	11.2[a]	2.09[b]	6.8

[a,b]: Within a column and within a trial, values with a different superscript are significantly different ($p < 0.05$).
(1): In trials 1 and 3, data show daily feed intake and in trials 2 and 4, overall feed intake.

3.3.3. Efficacy for laying hens

Three long-term trials were submitted. The details of the study design are provided in Table 4 and the results in Table 5. In the three studies the hens were fed either a non-supplemented diet (control) or a diet containing Belfeed B xylanase at the intended dose of 10–10.5 IU/kg feed (confirmed by analysis). In the three trials xylanase was added in liquid and solid forms. In trial 2, two breeds (Lohmann brown, LB; Lohmann selected light, LSL) of hens were used. Hens were under study for at least 24 weeks, and feed intake and laying performance were measured on weekly intervals in trial 1 or on 4-week intervals in trials 2 and 3. Feed to egg mass ratio was calculated. Health and mortality were monitored throughout the study. The results of laying rate, egg mass and feed to gain ratio were analysed with an ANOVA, cage basis, and comparison of group means was performed with the Tukey test.

Table 4: Trial design and dosages of the efficacy trials performed in laying hens

Trial	Total no. animals (cage/replicate)(d) Replicates/treatment	Breed (age at start) Duration	Diet composition	Enzyme activity (IU/kg)	
				Intended	Analysed
1(a)	1,152 (16 cages) 6	ISA Brown (26 weeks) 26 weeks	Wheat, corn, soya bean	0 10.5 solid 10.5 liquid	0 11.5 11.0
2(b)	216/216 (4 cages) 36 (18 LB/18 LSL)	LB/LSL (44 weeks) 24 weeks	Wheat, rye, soya bean	0 10.5 solid 10.5 liquid	0 12.3 10.8
3(c)	432 (6 cages) 8	Brown (22 weeks) 24 weeks	Wheat, rye, soya bean	0 10 liquid 10 solid	0 9.9 8.0

(a): Technical dossier/Section IV/Annex 04.01 and Supplementary information June 2013/Annex Q25-1 and Q27-2.
(b): Technical dossier/Section IV/Annex 04.02 and Supplementary information June 2013/Annex Q25-1 and Q27-2.
(c): Technical dossier/Section IV/Annex 04.03 and Supplementary information June 2013/Annex Q25-1 and Q27-2.
(d): Four hens per cage in trial 1, one hen per cage in trial 2 and three hens per cage in trial 3.

Xylanase resulted in a better feed to egg mass ratio in three trials (in trial 2 only in the LB strain and solid form); positive effects were also seen when incorporated in liquid form in trial 1 on the laying rate and in trials 1, 2 (only in the LSL strain) and 3 on the daily egg mass per hen. Therefore, the Panel concludes that the additive is efficacious in laying hens at the dose of 10 IU/kg feed.

Table 5: Effect of Belfeed B xylanase on the performance of laying hens

Trial	Treatments	Strain	Daily feed intake (g)	Laying rate (%)	Daily egg mass per hen (g)	Feed to egg mass	Mortality (%)
1	0		116	93.1[a]	60.8[b]	1.91[a]	2.3
	10.5 solid		114	93.2[a]	60.8[b]	1.88[b]	1.8
	10.5 liquid		115	93.7[b]	61.4[a]	1.88[b]	3.1
2	0	LSL	121	94.0	63.8[b]	2.02	0
	10.5 solid		118	93.0	64.6[ab]	1.99	0
	10.5 liquid		120	92.8	64.8[a]	2.00	0
	0	LB	128	93.0	68.4	2.02[a]	0
	10.5 solid		124	92.8	69.0	1.96[b]	1.3
	10.5 liquid		125	91.5	67.2	2.06[a]	0
3	0		115	94.2	56.6[b]	2.03[a]	2.6
	10.5 solid		113	94.2	56.9[b]	1.99[b]	1.8
	10.5 liquid		114	95.0	57.8[a]	1.97[b]	0.0

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

3.3.4. Efficacy for ducks and other minor poultry species

The applicant submitted two efficacy studies in ducks which had been previously evaluated by the FEEDAP Panel (EFSA, 2006).[57] The Panel considers that the mode of action of xylanase is well known and is considered to be similar within avian species. Therefore, the conclusions on the efficacy in chickens for fattening and laying hens can be extrapolated to minor poultry species for fattening and or laying/breeding when used at the same dose.

3.3.5. Efficacy for weaned piglets

A total of 12 trials were submitted (including the tolerance trial). Eight of these studies were not considered further in the assessment due to the short duration (from 36 to 41 days, shorter than the required 42 days),[58] the parameters measured (i.e. digestibility)[59] or the unavailability of relevant data/information.[60]

The details of the study design of the other four studies are provided in Table 6 and the results in Table 7. The four studies were performed with weaned piglets and included groups with a non-supplemented diet (control) or with a diet containing the Belfeed B xylanase at 10 IU/kg feed (confirmed by analysis). Trial 2 is the tolerance trial, described in the safety for the target species section, had also a third group with Belfeed B xylanase (tolerance dose). The Panel notes that in three of these trials (trials 2–4 below) the piglets received for at least 5 days medicated feed before the start of the trial (medication included amoxicillin, colistin and zinc oxide).[61] In the four trials, males and females were used and in trial 1, the animals were distributed to the pens according to sex. Health and mortality were monitored throughout the study, and body weight and feed intake were recorded. Feed to gain ratio was calculated. The data were analysed with an ANOVA. No significant differences were observed in any of the trials with the supplementation of Belfeed B xylanase at the dose of 10 IU/kg feed compared to control.

The data from the four studies were pooled and analysed statistically; differences were considered significant at a level of at least $p < 0.05$.[62] The groups studied included the control diet and the group with 10 IU/kg feed. The parameters studied included feed intake, final body weight, daily weight gain and feed to gain ratio. Data were tested for normality and homogeneity. Daily feed intake and final body weight data were not homogeneous and were not further considered in the analysis. An ANOVA was performed for the average daily weight gain and the feed to gain ratio, pen basis. The model included the effect of trial, treatment and their interaction, and the initial body weight was used as a

[57] Technical dossier/Section IV/Annex 03.01 and 03.02.
[58] Technical dossier/Section IV/Annexes 05.01, 05.02, 05.03, 05.06, 05.07 and 05.08.
[59] Technical dossier/Section IV/Annex 05.04.
[60] Technical dossier/Section IV/Annex 05.05.
[61] Technical dossier/Supplementary information June 2016.
[62] Technical dossier/Supplementary information January 2016.

covariate. Results showed a significant improvement in the feed to gain ratio in animals receiving 10 IU/kg compared to the control diet (1.36 vs 1.34) while no effect was observed on the average daily weight gain.

Table 6: Trial design and dosages of the efficacy trials performed in weaned piglets

Trial	Total no. animals (animals/replicate) replicates/group	Breed Sex Age at start	Diet composition	Enzyme activity (IU/kg)	
				Intended	Analysed
1[(a)]	288 (12) 12	Pietrain × (Landrace × Large White) ♀,♂ 28 days	Wheat, barley, soya bean meal	0 10	0 10.3
2[(b)]	144 (6) 8	Pietrain × (Landrace × Large White) ♀,♂ 28 days	Wheat, milk whey, rye, barley, soya bean meal	0 10 1,000	0 8 731
3[(c)]	72 (4) 9	Pietrain× (Duroc × Landrace) ♀,♂ 26 days	Wheat, milk whey, rye, barley, soya bean meal	0 10	0 11
4[(a)]	96 (4) 12	Pietrain × (Landrace × Large White) ♂,♀ 26 days	Wheat, milk whey, rye, barley, soya bean meal	0 10	0 7.5

(a): Technical dossier/Supplementary information January 2016/Annex 3.03_2015.
(b): Technical dossier/Supplementary information January 2016/Annex 3.03_2012.
(c): Technical dossier/Supplementary information January 2016/Annex 3.03_2013.

Table 7: Effects of Belfeed B xylanase on the performance of weaned piglets

Trial	Enzyme activity (IU/kg)	Daily feed intake (kg)	Body weight (kg)		Daily body weight gain (kg)	Feed to gain ratio	Dead and culled (%)
			Initial	Final			
1	0	0.54	7.9	24.2	0.37	1.40	1.4
	10	0.52	7.8	23.6	0.36	1.38	2.8
2	0	0.57	6.7	25.3	0.44	1.29[a]	8.3
	10	0.57	6.7	25.5	0.45	1.28[a]	6.2
	100	0.56	6.7	25.7	0.45	1.23[b]	4.1
3	0	0.79	9.0	33.8	0.58	1.37	5.5
	10	0.77	9.0	33.2	0.56	1.37	8.3
4	0	0.56	7.1	24.4	0.41	1.36	8.3
	10	0.54	7.1	24.1	0.41	1.34	2.1

[a,b]: Values within one column for the same study with different superscripts are different ($p < 0.05$).

Based on the results of the pooled data, which showed an improvement of the feed to gain ratio of the piglets, the Panel concludes that the additive has some potential to be efficacious in weaned piglets at the dose of 10 IU/kg feed.

3.3.6. Efficacy for pigs for fattening

Seven trials were assessed. The details of the studies are provided in Table 8 and the results in Table 9. The studies were performed with pigs for fattening which were treated either with a non-supplemented diet (control) or with a diet containing Belfeed B xylanase (solid or liquid) at an intended dose of 10.5 IU/kg feed. The analysis of the feed showed that the intended dose was equal or lower in most of the cases and therefore the dosage the animals received was not higher than the dose that the applicant recommends (10 IU/kg feed). The duration of the study was at least 10 weeks. The animals raised in groups were sex separated in trials 1, 2, 6 and 7. All trials except 5 and 6 were done by raising the pigs to a certain body weight. Health and mortality were monitored throughout the study, and body weight and feed intake were recorded. Feed to gain ratio was calculated. An ANOVA was performed with the data. No significant differences between the two dietary groups were found in relevant parameters.

Table 8: Trial design and dosages of the efficacy trials performed in pigs for fattening

Trial	Total no. animals (animals/replicate) Replicates/treatment	Breed Sex	Diet composition	Enzyme activity (IU/kg)	
				Intended	Analysed
1[(a)]	80 (10) 4	Pietrain × Hybrid ♂castrated, ♀	Wheat, barley, tapioca meal	0 10.5	0 7.9–9.9
2[(b)]	72 (6) 6	Pietrain × Hybrid ♂castrated, ♀	Wheat, barley, peas	0 10.5	0 10.1–10.4
3[(c)]	80 (10) 4	Pietrain × Hybrid ♂castrated, ♀	Wheat, peas, gluten feed	0 10.5	0 8.8–9.2
4[(d)]	46 (1) 23	Crossbred ♂castrated, ♀	Rye, barley, soya bean meal	0 10.5	0 10.6–11.9
5[(e)]	56 (1) 28	Crossbred ♂castrated, ♀	Wheat, barley	0 10.5	0 8.0–8.2
6[(f)]	80 (5) 8	Crossbred ♂, ♀	Wheat, barley	0 10.5	0 9.3–8.5
7[(g)]	60 (5) 6	Crossbred ♂castrated, ♀	Wheat, barley, peas	0 10.5	0 9.1–9.9

(a): Technical dossier/Section IV/Annex 06.01 and supplementary information June 2013.
(b): Technical dossier/Section IV/Annex 06.02 and supplementary information June 2013.
(c): Technical dossier/Section IV/Annex 06.03 and supplementary information June 2013.
(d): Technical dossier/Section IV/Annex 06.04 and supplementary information June 2013.
(e): Technical dossier/Section IV/Annex 06.05 and supplementary information June 2013.
(f): Technical dossier/Section IV/Annex 06.06 and supplementary information June 2013.
(g): Technical dossier/Section IV/Annex 06.07.

Table 9: Effects of Belfeed B xylanase on the performance of pigs for fattening

Trial	Enzyme activity (IU/kg)	Daily feed intake (kg)	Body weight (kg)		Daily weight gain (kg)	Feed to gain ratio	Mortality and culled (n)
			Initial	Final			
1	0	1.72	23	100	0.65	2.65	0
	10.5	1.75	23	100	0.66	2.65	1
2	0	2.05	19	107	0.71	2.88	2
	10.5	2.02	19	108	0.73	2.75	0
3	0	2.04	22	111	0.69	2.99	1
	10.5	2.02	22	111	0.68	2.97	1
4	0	2.57	27	107	0.84	3.04	1
	10.5	2.58	28	107	0.86	2.98	3
5	0	2.29	26	113	0.78	2.92	0
	10.5	2.31	26	116	0.80	2.88	0
6	0♂	2.28	43	108	0.95	2.39	2
	10.5♂	2.37	43	110	0.98	2.42	1
	0♀	2.22	43	99	0.82	2.70	1
	10.5♀	2.29	43	104	0.89	2.57	3
7	0	2.17[b]	29	112	0.83	2.62	4
	10.5	2.28[a]	30	111	0.88	2.61	3

[a,b]: Values within one column for the same study with different superscripts are different ($p < 0.05$).

The data from the seven studies were pooled and analysed statistically; differences were considered significant at a level of at least $p < 0.05$.[63] The groups studied included the control diet and the group with the intended dose of 10.5 IU/kg feed. The parameters studied included feed intake, final body weight, daily weight gain and feed to gain ratio. Data were tested for normality and homogeneity. When a study had an impact on the homogeneity of the data it was removed from the statistical analysis, so for final body weight the seven trials were included; for average daily gain, trials 1, 2, 4, 5 and 6 were included and for feed to gain ratio, trials 3, 4, 5 and 6 were included. An ANOVA was performed with the data considering two models (with or without the initial body weight as a covariate) which took into account the effect of the treatment, the effect of the trial, sex and their interactions. In all trials except trials 5 and 6, the animals remained under study up to a certain body weight; therefore, the analysis of the final body weight is of limited relevance for the efficacy. The results showed that the supplementation of the feed with Belfeed B xylanase improved significantly the average daily weight gain of the pigs (769 vs 791 g/day for control and Belfeed B xylanase).

Based on the results of the pooled data, which showed an improvement of the average daily weight gain of the pigs for fattening, the Panel concludes that the additive has some potential to be efficacious in pigs for fattening at the dose of 10 IU/kg feed.

3.3.6.1. Conclusions on efficacy for the target species

Based on the results obtained in the efficacy trials provided, the Panel concludes that the additive has the potential to be efficacious at the dose of 10 IU/kg feed in chickens for fattening, laying hens, weaned piglets and pigs for fattening. The Panel considers that this conclusion can be extended to chickens reared for laying and to breeding hens.

The Panel considers that the mode of action of xylanase is well known and is considered to be similar within avian species. Therefore, the conclusions on the efficacy in chickens for fattening and laying hens can be extrapolated to minor poultry species for fattening and or laying/breeding when used at the same dose.

Owing to the lack of sufficient data, the Panel cannot conclude on the efficacy of the product in turkeys for fattening.

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[64] and good manufacturing practice.

4. Conclusions

Belfeed B does not give rise to safety concerns with regard to the genetic modification of the production strain. Neither the production strain nor its recombinant DNA was detected in the final product.

The additive is safe for chickens and turkeys for fattening, laying hens and weaned piglets at the dose of 10 IU/kg feed. The Panel considers that the conclusions can be extended to chickens reared for laying, turkeys reared for breeding, breeding hens and pigs for fattening. Also and based on the wide margin of safety shown in major poultry species, the Panel considers that the conclusions can be extrapolated to minor poultry species at the same dose.

The use of Belfeed B as a feed additive does not give rise to concerns for consumers.

The additive is not considered to be toxic by inhalation or irritant for skin or eye, but the Panel cannot conclude on its skin sensitisation potential. Owing to the proteinaceous nature of the active substance, the additive is considered a potential respiratory sensitiser.

The use of Belfeed B as a feed additive poses no risks to the environment.

The additive has the potential to be efficacious at the dose of 10 IU/kg feed in chickens for fattening, laying hens, weaned piglets and pigs for fattening. The Panel considers that this conclusion can be extended to chickens reared for laying and to breeding hens and can also be extrapolated to

[63] Technical dossier/Supplementary information January 2016/Annex 2.

[64] 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.

minor poultry species for fattening and or laying/breeding. Owing to the lack of sufficient data, the Panel cannot conclude on the efficacy of the product in turkeys for fattening.

Documentation provided to EFSA

1) Belfeed B for poultry and pigs. November 2010. Submitted by Beldem.
2) Belfeed B for poultry and pigs. Supplementary information. June 2013. Submitted by Beldem.
3) Belfeed B for poultry and pigs. Supplementary information. March 2014. Submitted by Beldem.
4) Belfeed B for poultry and pigs. Supplementary information. September 2014. Submitted by Beldem.
5) Belfeed B for poultry and pigs. Supplementary information. November 2014. Submitted by Beldem.
6) Belfeed B for poultry and pigs. Supplementary information. January 2016. Submitted by Beldem.
7) Belfeed B for poultry and pigs. Supplementary information. June 2016a. Submitted by Beldem.
8) Belfeed B for poultry and pigs. Supplementary information. June 2016b. Submitted by Beldem.
9) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Belfeed B.
10) Comments from Member States.

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Abbreviations

ANOVA	analysis of variance
CFU	colony forming unit
EC	European Commission
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization of the United Nations
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
ME	metabolisable energy
OECD	Organisation for Economic Co-operation and Development
QPS	Qualified Presumption of Safety

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Belfeed B MP and Belfeed B ML

In the current application authorisation is sought under articles 4(1); 10(2) and 13(3) for *Belfeed B MP* and *Belfeed B ML* under the category/functional 4(a) 'zootechnical additives'/'digestibility enhancers'. Specifically, authorisation is sought for the use of the *feed additive* for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding and for breeding purposes, laying hens, other ducks, other minor poultry species for fattening and laying, weaned pigs and pigs for fattening.

According to the Applicant, *endo-1,4-β-xylanase* (EC 3.2.1.8) is the active agent of *Belfeed B MP & ML* produced by *Bacillus subtilis* - TD160(229) strain. The Applicant expresses the *xylanase* enzymatic activity in international unit (IU), defined as 'the amount of enzyme which liberates one micromole of reducing sugars (xylose equivalents) from beechwood xylan per minute at pH 4.5 and 30°C'.

The product is intended to be marketed as granulated and liquid formulations having a guaranteed minimum *xylanase* activity of 400 IU/g. The carrier in the solid formulation is wheat flour, while glycerol and acetate buffer are 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 10 IU/kg in *feedingstuffs* for all the target species. For the quantification of the *xylanase* activity in the *feed additive* and *premixtures* the Applicant proposed the single laboratory validated 'Skalar method', a method that according to the Applicant is only available in his laboratory. Upon request by the EURL the Applicant submitted another single-laboratory validated and further verified colorimetric method for the Quantification of xylanase activity in the *feed additive*, 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 50°C (DNS method). The use of a reference sample, consisting on a reference enzyme standard with a certified enzymatic activity expressed in international units (IU), analysed in parallel with the *feed additive* samples when determined using the DNS method, ensures the measurement traceability. Based on the performance characteristics recalculated from the experimental data available the EURL recommends for official control the proposed single-laboratory validated and further verified colorimetric method above mentioned for the quantification of the xylanase activity in the feed additive.

For the quantification of the *xylanase* activity in *feedingstuffs*, the Applicant proposed a second colorimetric method, based on the quantification of water soluble dyed fragments produced by the action *xylanase* on commercially available azurine cross-linked arabinoxylan substrates. The calibration is conducted using a reference standard with a known enzyme activity expressed in IU. Upon request of the EURL, the Applicant performed the analysis of *premixture* samples after a solid dilution with a blank feed and applied the above mentioned method developed for *feedingstuffs*. Based on the satisfactory performance characteristics recalculated from the experimental data available the EURL recommends for official control the proposed single-laboratory validated and further verified colorimetric method for the quantification of the *xylanase* activity in *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.