Safety and efficacy of FRA® Octazyme C Dry 
(α-galactosidase, α-amylase, endo-1,3(4)-β-glucanase, 
endo-1,4-β-glucanase, mannan-endo-1,4-β-mannosidase, 
pectinase, protease, endo-1,4-β-xylanase) for chickens 
for fattening and weaned piglets

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Abstract

FRA® Octazyme C Dry is a product that presents α-galactosidase, α-amylase, endo-1,3(4)-β-glucanase, 
endo-1,4-β-glucanase, mannan-endo-1,4-β-mannosidase, pectinase, protease and endo-1,4-β- 
xylanase. It is intended to be used as a zootechnical additive for chickens for fattening and weaned piglets. The enzymes present in the additive are obtained from six different fermentation processes with four different microorganisms, Trichoderma citroviride, Aspergillus niger, Bacillus licheniformis and Bacillus amyloliquefaciens. In the tolerance and efficacy trials done in the target species, major limitations on the analysed enzyme activities in the feed were identified. These limitations did not permit the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) to conclude on the safety and efficacy of FRA® Octazyme C Dry for the target species. The Panel concluded that the additive raises no concerns for the consumers of food products obtained from animals fed with the additive. No specific data were submitted to address the safety for the user, in the absence of such data the FEEDAP Panel could not conclude on the potential of the additive to be irritant to the skin and eyes or on its skin sensitising properties. Owing to the nature of the active substances, the additive should be considered a potential respiratory sensitiser. The use of FRA® Octazyme C Dry as a feed additive poses no risks to the environment.

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Amendment: This scientific opinion has been amended following the adoption of the decision of the Commission on confidentiality claims submitted by the applicant, in accordance with Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The modified sections are indicated in the text.

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Summary

Following a request from European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on safety and efficacy of FRA® Octazyme C Dry (α-galactosidase, α-amylase, endo-1,3(4)-β-glucanase, endo-1,4-β-glucanase, mannan-endo-1,4-β-mannosidase, pectinase, protease, endo-1,4-β-xylanase) for chickens for fattening and weaned piglets. The enzymes present in the additive are obtained from six different fermentation processes with four different microorganisms, Trichoderma citroviride, Aspergillus niger, Bacillus licheniformis and Bacillus amyloliquefaciens.

In order to support the safety for the target species, the applicant submitted one tolerance trial in chickens for fattening and another one in weaned piglets. However, limitations on the analysed enzyme activities in the feed were found in the two studies. These limitations did not permit the FEEDAP Panel to conclude on the safety of FRA® Octazyme C Dry for the target species.

Toxicological studies are not required for products obtained from microorganisms belonging to species that are considered suitable for the qualified presumption of safety (QPS) approach for safety assessment provided that the qualifications are met. The fermentation products containing the protease and the amylase are obtained from B. licheniformis and from B. amyloliquefaciens, respectively. Considering the information provided on the production strains, the Panel concluded that those products do not raise concerns for consumer safety. The results obtained in the genotoxicity studies and in the subchronic oral toxicity studies for the fermentation products obtained from T. citroviride and A. Niger did not indicate any reason for concern. Therefore, the Panel concluded that the additive raise no concerns for the consumers of food products obtained from animals fed with the additive.

No specific data were submitted to address the safety for the user; in the absence of such data, the FEEDAP Panel could not conclude on the potential of the additive to be irritant to the skin and eyes or on its skin sensitising properties. Owing to the nature of the active substances, the additive should be considered a potential respiratory sensitisier.

The applicant submitted three efficacy trials in chickens for fattening and three in weaned piglets. However, limitations on the analytical confirmation of the enzyme activities in the diets were identified. These limitations did not permit the FEEDAP Panel to conclude on the efficacy of the product.

The use of FRA® Octazyme C Dry as a feed additive poses no risks to the environment.
1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/20031 establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Framelco B.V.2 for authorisation of the product FRA® Octazyme C Dry (α-galactosidase, α-amylase, endo-1,3(4)-β-glucanase, endo-1,4-β-glucanase, mannan-endo-1,4-β-mannosidase, pectinase, protease, endo-1,4-β-xylanase), when used as a feed additive for chickens for fattening and weaned piglets (category: zootechnical additives; functional group: digestibility enhancers).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 24 July 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 FRA® Octazyme C Dry (α-galactosidase, α-amylase, endo-1,3(4)-β-glucanase, endo-1,4-β-glucanase, mannan-endo-1,4-β-mannosidase, pectinase, protease, 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 dossier3 in support of the authorisation request for the use of FRA® Octazyme C Dry (α-galactosidase, α-amylase, endo-1,3(4)-β-glucanase, endo-1,4-β-glucanase, mannan-endo-1,4-β-mannosidase, pectinase, protease, endo-1,4-β-xylanase) as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/20084 and the applicable EFSA guidance documents.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substances 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 FRA® Octazyme C Dry is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c), Guidance on the assessment of the toxigenic potential of Bacillus species used in animal nutrition (EFSA FEEDAP Panel, 2014), Technical Guidance: Microbial Studies (EFSA, 2008b), Technical

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1 Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
2 Framelco B.B., Ruisvoorn 5 4941 SB Raamsdonksweer, Netherlands.
3 FEED dossier reference: FAD-2014-0028.
4 Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
5 The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/fnirep-fad-2014-0028-fraoctazymec.pdf
Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008c), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel 2012d), and Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011).

3. Assessment

FRA® Octazyme C Dry is an enzyme preparation intended to be used in feed for chickens for fattening and weaned piglets as a zootechnical additive (functional group: digestibility enhancers).

3.1. Characterisation

3.1.1. Characterisation of the active substances

FRA® Octazyme C Dry contains the following declared activities: endo-1,4-β-xylanase (Enzyme Commission number (EC) 3.2.1.8; xylanase), endo-1,4-β-glucanase (EC 3.2.1.4; cellulase), endo-1,3 (4)-β-glucanase (EC 3.2.1.6; glucanase), mannan-endo-1,4-β-mannosidase (EC 3.2.1.78; mannanase), α-galactosidase (EC 3.2.1.22; galactosidase), pectinase, protease (EC 3.4.21.62) and α-amylase (EC 3.2.1.1; amylase).

3.1.1.1. Characterisation of the production strains

The xylanase, cellulase, glucanase and mannanase are produced by a strain of *Trichoderma citrinoviride* which is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) with the deposition number DSM 27790. The strain has not been genetically modified and has been identified as *T. citrinoviride* by sequence analysis of the ITS1-ITS2 of the ribosomal operons and partial segment of elongation factor 1α gene. Data showing its genetic stability were provided. The strain was found not to produce the following secondary metabolites in laboratory conditions: deoxynivalenol (DON), 3-en 15-acetyl-DON, aflatoxins (B1, B2, G1 and G2), fumonisins (B1, B2 and B3), HT-2, T-2, A-zearalenol, B-zearalenol, zearalenol, agroclavine, alternariol, alernariol-methyl ether, beauvericin, citrinin, diacetoxyscirpenol, moniliformin, mycophenolic acid, nitropropionic acid, roquefortine C, sterigmatocystin.

The galactosidase and the pectinase are produced by a strain of *A. niger* which is deposited in the DSMZ with the deposition number DSM 27958. The strain has not been genetically modified and it has been identified by sequence analysis of the partial β-tubulin and calmodulin genes, and ITS region of the ribosomal operons. Data showing its genetic stability were provided. The strain was found not to produce fumonisins (B1–B3) or ochratoxin A in laboratory conditions.

The protease is produced by a strain of *Bacillus licheniformis* which is deposited in the DSMZ with the deposition number DSM 27949. The strain has not been genetically modified and has been identified biochemically and by 16S rRNA gene sequence analysis. Data showing its genetic stability were provided. The strain was found not to be haemolytic or cytotoxic to Vero cells according to the recommendations made by EFSA (EFSA FEEDAP Panel, 2014). The susceptibility of the strain to the antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2012d) was tested and all the minimum inhibitory concentration (MIC) values were below the corresponding cut-off values defined by the FEEDAP Panel.

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6 This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
7 Technical Dossier/Supplementary information August 2016/Annex B2.2.
8 Technical Dossier/Section II/Annex 2.2.1.2.d.
9 Technical Dossier/Supplementary information October 2015.
10 Technical Dossier/Section II/Annex 2.2.1.2.e.
11 Technical Dossier/Section II/Annex 2.2.1.2.f.
12 Technical Dossier/Section II/Annex 2.2.1.2.a.
13 Technical Dossier/Section II/Annex 2.2.1.2.b.
14 Technical Dossier/Section II/Annex 2.2.1.2.k.
15 Technical Dossier/Section II/Annex 2.2.1.2.j.
16 Technical dossier/Supplementary information August 2016/Annex B1.3.
17 Technical Dossier/Section II/Annex 2.2.2.2.d.
18 Technical Dossier/Section II/Annex 2.2.2.2.f.
19 Technical dossier/Supplementary information August 2016/Annex B1.5.
The amylase is produced by a strain of *Bacillus amyloliquefaciens* which is deposited in the DMSZ with the deposition number DSM 27947. The strain has not been genetically modified and has been identified biochemically and by 16S rRNA gene sequence analysis; however, the results do not enable to distinguish whether the strain should be identified as *B. amyloliquefaciens* or *Bacillus subtilis* (99% identity with both species). Data showing its genetic stability were provided. The strain was found not to be haemolytic or cytotoxic to Vero cells according to the recommendations made by EFSA (EFSA FEEDAP Panel, 2014). The susceptibility of the strain to the antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2012a–d) was tested and all the MIC values were below the corresponding cut-off values defined by the FEEDAP Panel.

### 3.1.2. Manufacturing

The enzymes are produced by fermentation with the production strains. The xylanase, cellulase and glucanase are produced in the same fermentation process, whereas the rest of the enzymes are produced independently. Detailed information on the respective fermentation processes, purification and concentration steps of the enzymes as well as on the preparation of the final formulation was provided and it was considered sufficient by the FEEDAP Panel.

### 3.1.3. Characterisation of the additive

FRA® Octazyme C Dry is a solid formulation which contains the enzyme mixtures, calcium propionate, vermiculite and sepiolite.

This formulation aims to ensure a minimum activity of 160,000 BXU of xylanase, 1,000 Uₘ of mannanase, 10,000 Uₐ of amylase, 20,000 BU of glucanase, 1,500 Uₚ of protease, 3,200 Uₙ of cellulase, 2,100 UP of pectinase and 80 GALU of galactosidase per gram of product. The definition of the enzyme units is provided in Annex A.

The study of the batch-to-batch variation submitted in the initial dossier for five batches showed major deviations from the specifications of the additive. For some enzymes, the activities were below the specifications (two batches for mannanase, amylase and protease and four batches for cellulase), while others showed an average higher than a 30% (two batches for amylase, three for galactosidase and five for xylanase, glucanase and pectinase). The applicant provided supplementary data obtained from five different batches (same day of preparation) and analysed by two independent laboratories one month after production. The mean enzyme activities per gram of product were (range and coefficient of variation (CV, %)): 188,948 BXU of xylanase (173,396–201,001; 5%), 1,065 Uₘ of mannanase (1,022–1,105; 4%), 10,819 Uₐ of amylase (9,956–11,274; 5%), 22,568 BU of glucanase (21,638–24,062; 4%), 1,417 Uₚ of protease (1,304–1,486; 5%), 3,598 Uₙ of cellulase (3,498–3,684; 2%), 2,485 UP of pectinase (2,397–2,548; 2%) and 80 GALU of galactosidase (74–88; 6%). The ratio analysed:specified was 1.26 for xylanase, 1.0 for amylase, protease and galactosidase, 1.20 for mannanase and cellulose and 1.20 for glucanase and pectinase. Therefore, compliance with the specifications of the additive was shown.

The additive has a bulk density of 650 kg/m³. Particle size and dusting potential was studied in three batches of the additive. Particles below 100 μm amount 44%, below 50 μm amount 30% and below 10 μm amount less than 13%. Dusting potential of three batches, measured according to Stauber–Heubach, ranged from 1.6 to 2.2 g/m³. Three batches of each of the two enzyme mixtures used to prepare the additive, SEB Multizyme and SEB 150, were analysed for microbiological and chemical contamination. Microbiological analysis included *Salmonella* spp. and *Escherichia coli* (absence in 25 g and 10 g, respectively), total

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20 Technical Dossier/Section II/Annex 2.2.1.2.m.
21 Technical Dossier/Section II/Annex 2.2.1.2.l.
22 Technical dossier/Supplementary information August 2016/Annex B1.4.
23 Technical Dossier/Section II/Annex 2.2.2.2.c.
24 Technical Dossier/Section II/Annex 2.2.2.2.e.
25 Technical dossier/Supplementary information August 2016/Annex B1.6.
26 Technical Dossier/Section II/Annexes 2.3.1.a to 2.3.1.e and Supplementary information August 2016/Annex B4.
27 Technical Dossier/Section II/Annex 2.1.3.b and Supplementary information August 2016.
28 Technical dossier/Supplementary information August 2016/Annex A1.6 and A1.7.
29 Technical dossier/Supplementary information August 2016/Annex B10.14.
30 Technical dossier/Section II/Table 2.1.5.a and Annexes 2.1.4.e1-2.1.4.e3.
31 Technical dossier/Section II/Annex 2.4.3 and supplementary information August 2016.
32 Technical dossier/Section II/Annex 2.4.1.c and 2.4.1.d.
yeast and moulds (< 10 CFU/g) and total coliforms (only in SEB Multizyme, absent). The absence of moulds in SEB Multizyme may indicate the absence of the production strains *T. citroviride* and *A. niger*. Data supporting the absence of the production strains *B. licheniformis* and *B. amyloliquefaciens* (in SEB Multizyme) was not provided but it is noted that the manufacturing of the product includes filtration steps aimed at removing them, and therefore the absence can be reasonably assumed. The analyses of chemical contamination included arsenic (< 0.1 mg/kg), cadmium (< 0.01 mg/kg), lead (1.18 and 0.95 mg/kg, respectively) and mercury (< 0.1 mg/kg). The following mycotoxins were not detected: aflatoxin B1, B2, G1, G2, M1, ochratoxin A, fumonisin B1, zearalenone, DON, nivalenol (only in SEB 150), T2-toxin, HT2-toxin, ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine.

Moreover, three batches of FRA® Octazyme C Dry were checked for the potential presence of mycotoxins and secondary metabolites based on a list of 652 available compounds and only the following mycotoxin/secondary metabolites were found: DON (10.0–13.1 µg/kg), 3-nitropropionic acid (1.6–2 µg/kg), brevianamid F (92.1–114 µg/kg), cyclo (L-Pro-L-Tyr) (762–973 µg/kg), cyclo (L-Pro-L-Val) (439–593 µg/kg), emodin (0.7–0.8 µg/kg), neoechinulin A (1.6–2.8 µg/kg), rugulosavin (7.4–9.6 µg/kg), tryptophol (22.8–50.3 µg/kg). Three batches of FRA® Octazyme C Dry were also analysed for polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (WHO-TEQ ≤ 0.137 ng/kg), PCBs (WHO-TEQ 0.140 ng/kg) and PCDD/F-PCB (WHO TCQ ≤ 0.280 ng/kg).

The absence of antimicrobial activity was studied in three batches of each of the premixtures SEB Multizyme and SEB 150 by using eight bacterial strains as proposed in the EFSA guidance on microbial studies (EFSA, 2008a,b,c). No antibacterial activity was found.

### 3.1.4. Stability and homogeneity

The shelf-life of FRA® Octazyme C Dry was studied in samples from three batches stored in closed containers for up to 12 months at 25°C or up to 6 months at 40°C. Recoveries varied depending on the enzyme and storage conditions. In samples stored for 12 months at 25°C, recoveries of the initial enzyme activity varied between 41% (pectinase) and 160% (mannanase) with an average of 92%. In samples stored for 6 months at 40°C, recoveries varied between 15% (pectinase) and 159% (xylanase).

The stability of FRA® Octazyme C Dry when added to a vitamin and mineral premixture (not containing choline chloride) was studied for three batches of the additive. The addition to the premixture was at a dose of 5 g/kg and samples were stored up to 6 months at room temperature in closed containers. Recoveries after 6 months storage ranged between 65% (amylase) and 90% (galactosidase), mean recovery (excluding protease for which data at 6 months could not be evaluated) was 74%.

The stability of FRA® Octazyme C Dry in feed was studied in three batches of the additive added at the recommended dose to three complete feeds (two for chickens for fattening, and one for weaned piglets). The feeds were subject to pelleting at 65–70°C. Samples of mash and pelleted feed were stored in close containers at 25°C for up to 3 months. The recovery values after heat treatment showed values ranging from 45% for amylase and galactosidase to 87% for xylanase. For the effect of storage data on mannanase, glucanase (only for the stability to storage), protease and cellulase were excluded from the study (because the method used could not detect/quantify (no explanations were given) the enzyme activity). The recoveries after 3 months of storage ranged in the mash feed from

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33 Technical dossier/Supplementary information August 2016.
34 Technical dossier/Section II/Annexes 2.1.4.f and 2.1.4.g the limits of detection were as follows: aflatoxin B1 (< 1 µg/kg), aflatoxin B2 (< 1 µg/kg), aflatoxin G1 (< 1 µg/kg), aflatoxin G2 (< 1 µg/kg), aflatoxin M1 (< 0.4 µg/kg), ochratoxin A (< 5 µg/kg), fumonisin B1 (< 100 µg/kg), zearalenone (< 5 µg/kg), DON (< 25 µg/kg), nivalenol (only in SEB 150, < 800 µg/kg), T2-toxin (< 10 µg/kg), HT2-toxin (< 50 µg/kg), ergocornine (< 100 µg/kg), ergocristine (< 100 µg/kg), ergocryptine (< 100 µg/kg), ergometrine (< 100 µg/kg), ergosine (< 100 µg/kg) and ergotamine (< 100 µg/kg).
35 Technical dossier/Supplementary information August 2016/Annex B11.
36 Technical dossier/Section II/Annexes 2.1.4.e1-2.1.4.e3.
37 Technical dossier/Section II/Annex 2.2.2.a and 2.2.2.b.
38 Technical dossier/Section II/Annexes 2.4.1.a1-2.4.1.a3 and Supplementary information August 2016/Annex B12.
39 Technical dossier/Section II/Annexes 2.4.1.b1-2.4.1.b3 and Supplementary information August 2016/Annex B12.
40 Technical dossier/Section II/Annexes 2.4.1.c4 and Supplementary information August 2016.
41 Technical dossier/Section II/Annexes 2.4.1.c1-2.4.1.c3 and Supplementary information August 2016.
42 Technical dossier/Section II/Annexes 2.4.1.f1-2.4.1.f3 and Supplementary information August 2016.
43 Technical dossier/Section II/Annexes 2.4.1.e1-2.4.1.e3 and Supplementary information August 2016.
51% (pectinase) to 96% (galactosidase) and in the pelleted feed from 61% (pectinase) to 130% (galactosidase).

One batch of FRA® Octazyme C Dry was added at 100 mg/kg feed to a complete compound feed.44 Ten subsamples were analysed only for galactosidase activity and the CV was calculated. The mean value of galactosidase activity was 500 GALU/kg feed with a coefficient of variation was 9%.

3.1.5. Conditions of use

FRA® Octazyme C Dry is intended to be used in feed for chickens for fattening and weaned piglets at a recommended dose of 50 mg additive per kg feed (delivering 8,000 BXU xylanase units, 50 UM mannanase, 500 Uₐ amylase, 1,000 BU glucanase, 75 Uₚ protease, 160 Uₚ cellulase, 105 Uₚ pectinase and 4 GALU galactosidase per kg feed).

3.2. Safety

3.2.1. Safety for the target species

The applicant submitted one tolerance trial in chickens for fattening and another one in weaned piglets.45 The two studies were designed considering a control, recommended use level and a 100-fold the recommended dose. The performance of the animals was measured throughout the study period; no other parameters were measured in the animals.

However, limitations on the analysed enzyme activities in the feed were found in the two studies. The analytical results for the feeds administered to the animals are presented in Appendix A (Table A.1). The results in the study done with chickens for fattening showed that (i) the control diet presented high enzyme activities, in some cases similar to the ones present in the feed supplemented at the recommended dose and (ii) the intended dosages were not confirmed by analysis (in the 100-fold diet mean enzyme activity was 26% of the intended dose). This fact limits the acceptability of the study because the measurements on the performance of the birds do not suffice to demonstrate the tolerance. In the case of the study done in piglets, the control diet46 showed enzyme activities higher than the feed containing the recommended dose, therefore, the study lacks a control and no conclusions can be drawn.

Due to the limitations in the studies provided, the FEEDAP Panel cannot conclude on the safety of FRA® Octazyme C Dry for the target species.

3.2.2. Safety for the consumer

Tests were provided for the fermentation products obtained from T. citroviride DSM 27790 and containing xylanase, glucanase and cellulase, T. citroviride DSM 27790 containing mannanase, A. Niger DSM 27958 containing pectinase and A. Niger DSM 27958 containing galactosidase. The studies under this section were done with the fermentation products that are used to formulate the additive.

3.2.2.1. Trichoderma citroviride DSM 27790 – xylanase, cellulase and glucanase

The fermentation product was tested by plate incorporation assay in Salmonella Typhimurium strains TA1535, TA1537, TA98, TA100 and TA102 both with and without microsomal enzyme activation (S9 mix) according to OECD Guideline 471.47 There was no evidence for genotoxicity of the test article when used up to 5 mg/plate. Positive controls performed as expected.

The enzyme preparation was tested for in vitro cytogenetic damage in cultured human lymphocytes both with and without microsomal enzyme activation (S9 mix) according to OECD guideline 473 up to a maximum concentration of 5 mg/mL.48 In the first experiment, cells were treated for 4 h with and without S9 mix, and in the second experiment, cells were treated for 4 h with S9 mix and for 24 h without. The test item did not induce any significant increase in the number of chromosome aberrations while the positive controls performed as expected.

A mouse micronucleus test was conducted in five Swiss albino mice per sex, given a single oral dose of 2,000 mg/kg body weight (by gavage) for two consecutive days in compliance with OECD...
The animals were sacrificed for bone marrow harvesting 24 h after the second treatment. There was no significant or dose-related increase in micronuclei in treated groups, while the positive control induced a clear response; however no evidence of target cell exposure (local cytotoxicity at bone marrow) was reported in this study.

The systemic toxic potential of the enzyme concentrate to rats by oral administration was assessed over a period of 13 weeks, in compliance with OECD guideline 408. Four groups of 10 Wistar rats of each sex received the test material by gavage (10 mL/kg body weight per day) for 90 days at doses of 0, 250, 500 or 1,000 mg/kg body weight per day. Two more groups received the test material at doses of 0 or 1,000 mg/kg body weight per day for 90 days and were then allowed to recover for a month. The control group received the vehicle (purified water). No animals died during the study and there were no differences in the recorded clinical observations between control and treated groups. Some statistical differences were found in the food intakes of treated groups in some of the weekly measurements but these differences were not consistently present and did not lead to differences between the groups in the mean body weight of the rats. No changes due to treatment were found in the ophthalmological examinations, sensory reactivity, motor activity or in the grip strength of the rats. In the blood chemistry analysis, the only observed differences were a lower alanine aminotransferase activity in the males treated with the highest dose both on day 91 and after 4 weeks without treatment, and a lower urea level in high-dose females on day 91. Although the results of the haematology examination showed a lower granulocyte count in the females of the highest dose, this difference was very small. No differences were seen in the organ weights of the different groups nor in gross or microscopic examination of tissues.

3.2.2.2. Trichoderma citroviride DSM 27790 – mannanase

The fermentation product was tested in Salmonella Typhimurium strains TA1535, TA97a, TA98, TA100 and TA102 both with and without microsomal enzyme activation (S9 mix), according to OECD Guideline 471. The test was conducted by the plate incorporation method up to a maximum concentration of 5 mg/plate. No increase in the frequency of revertant colonies was reported in any experimental condition while the positive controls performed as expected.

The clastogenic potential of the enzyme preparation was tested in cultured human lymphocytes in vitro both with and without microsomal enzyme activation (S9 mix) according to OECD guideline 473. In the first experiment, cells were treated for 4 h with and without S9 mix, and in the second experiment, cells were treated for 4 h with S9 mix and for 24 h without. The test item did not induce any significant increase in the number of chromosome aberrations up to a maximum concentration of 5 mg/mL. The positive controls performed as expected.

A mouse micronucleus test was conducted in compliance with OECD guideline 474 (version 1997). Five Swiss albino mice per sex were treated with a single oral dose of 2,000 mg/kg body weight (by gavage) for two consecutive days and sacrificed for bone marrow harvesting 24 h after the second treatment. No significant or dose-related increase in micronuclei in treated groups was observed, while there was a clear response in the positive control; however, no evidence of target cell exposure (local cytotoxicity at bone marrow) was reported.

The main subchronic oral toxicity study was preceded by a subacute oral toxicity study with the same strain of rats to check suitability of the doses used. The systemic toxic potential of the enzyme concentrate to rats by oral administration was assessed over a period of 13 weeks, in compliance with OECD guideline 408. Four groups of 10 Sprague–Dawley rats of each sex received the test material by gavage (10 mL/kg body weight per day) for 90 days at doses of 0, 100, 500 or 1,000 mg/kg body weight per day. Two more groups received the test material at doses of 0 or 1,000 mg/kg body weight per day for 90 days and then were allowed to recover for a month. The control group received the vehicle (purified water). No animals died during the study and there were no significant differences in the recorded clinical observations between control and treated groups. There were no significant effects of treatment on feed consumption or body weight gain, although the body weight of the highest dose group of both sexes was slightly lower than controls by the end of the study.

49 Technical dossier/Section III/Annex 3.2.2.2.c.
50 Technical dossier/Section III/Annex 3.2.2.3.a.
51 Technical dossier/Section III/Annex 3.2.2.2.g.
52 Technical dossier/Section III/Annex 3.2.2.2.f.
53 Technical dossier/Section III/Annex 3.2.2.2.d.
54 Technical dossier/Section III/Annex 3.2.2.2.h.
55 Technical dossier/Section III/Annex 3.2.2.3.e and Supplementary information August 2016 Annex B19.1.
study and this difference remained after the reversal period. No changes due to treatment were found in the ophthalmological examinations, sensory reactivity, motor activity or in the grip strength of the rats. The haematological results showed higher platelet levels compared with controls in high-dose male rats at the end of the study and after the reversal period; a similar difference was not seen in females. Other haematological differences were seen (lower mean corpuscular haemoglobin concentration and platelet levels in females) but these showed no relation to dose. The results of the blood chemistry analyses showed higher serum sodium levels in high-dose males after the reversal period and higher serum phosphorus levels in high-dose females after the reversal period. Urinalysis revealed no differences between control and treated groups. Although there were no differences in organ weights of males at the end of the study, the high-dose animals at the end of the recovery period had higher relative weights of brain, liver, kidneys, testes and heart compared with controls. High-dose females showed higher relative weights of brain and ovaries and lower relative adrenal weights at the end of the study. After the recovery period, relative weights of liver and spleen were higher for the high-dose group than those of controls. Gross and microscopic examination of tissues revealed no differences between high-dose and control groups.

3.2.2.3. *Aspergillus niger DSM 27958* – pectinase

The mutagenic potential of the fermentation product was tested in *Salmonella* Typhimurium strains TA1535, TA97a, TA98, TA100 and TA102 (plate incorporation assay) both with and without microsomal enzyme activation (S9 mix) according to OECD Guideline 471. There was no evidence for genotoxicity of the test article when used up to 5 mg/plate. Positive controls performed as expected.

The enzyme preparation was tested for *in vitro* cytogenetic damage in cultured human lymphocytes both with and without microsomal enzyme activation (S9 mix) according to OECD guideline 473 up to a maximum concentration of 5 mg/mL. In the first experiment, cells were treated for 4 h with and without S9 mix, and in the second experiment, cells were treated for 4 h with S9 mix and for 24 h without. The test item did not induce any significant increase in the number of chromosome aberrations. The positive controls performed as expected.

The clastogenic and aneugenic potential of the test item was tested in a mouse micronucleus test, in compliance with OECD guideline 474. The test was conducted in five Swiss albino mice per sex given a single oral dose of 2,000 mg/kg body weight (by gavage) for two consecutive days. Mice were sacrificed for bone marrow harvesting 24 h after the second treatment. No significant or dose-related increase in micronuclei in treated groups was observed, while there was a clear response in the positive control, however no evidence of target cell exposure (local cytotoxicity at bone marrow) was reported.

The systemic toxic potential of the enzyme concentrate to rats by oral administration was assessed over a period of 13 weeks, in compliance with OECD guideline 408. The study was preceded by a dose-range finding study. Four groups of 10 Sprague–Dawley rats of each sex received the test material by gavage (10 mL/kg body weight per day) for 90 days at doses of 0, 100, 500 or 1,000 mg/kg body per day. Two more groups received the test material at doses of 0 or 1,000 mg/kg body weight per day for 90 days and then were allowed to recover for a month. The control group received the vehicle (purified water). No animals died during the study and there were no differences in the recorded clinical observations between control and treated groups. There were no significant effects of treatment on feed consumption or body weight gain. No changes due to treatment were found in the ophthalmological examinations, sensory reactivity, motor activity or in the grip strength of the rats. The haematological results showed lower red blood cell (RBC) counts compared with controls in high-dose male rats at the end of the study and lower mean corpuscular haemoglobin concentration (MCHC) and platelets after the reversal period; similar differences were not seen in females. The results of the blood chemistry analyses showed a few differences which were unrelated to dose but higher serum sodium levels were found in intermediate and high-dose females and lower blood urea nitrogen in high-dose males. Urinalysis revealed no differences between control and treated groups. Although there were no differences in organ weights of males at the end of the study the high-dose animals at the end of the recovery period had higher relative weights of testes and heart compared with controls. No differences were seen in organ weights between female treated and control groups.
at any stage. Gross and microscopic examination of tissues revealed no differences between high-dose and control groups.

### 3.2.2.4. Aspergillus niger DSM 27958 – Galactosidase

The fermentation product was tested in *Salmonella* Typhimurium strains TA1535, TA97, TA98, TA100 and TA102 both with and without microsomal enzyme activation (S9 mix) according to OECD Guideline 471. No evidence of genotoxicity was observed up to a maximum concentration of 5 mg/plate. The positive controls performed as expected.

The enzyme preparation was tested for *in vitro* cytogenetic damage in cultured human lymphocytes both with and without microsomal enzyme activation (S9 mix) according to OECD guideline 473. Cells were treated for 4 h in the first experiment with and without S9 mix, and in the second experiment with S9 mix, while in the second experiment without S9 mix the treatment lasted 24 hours. The maximum concentration tested was 5 mg/mL. The test item did not induce any significant increase in the number of chromosome aberrations while positive controls performed as expected.

A mouse micronucleus test was conducted in five Swiss albino mice per sex, given a single oral dose of 2,000 mg/kg body weight (by gavage) for two consecutive days, in compliance with OECD guideline 474. Mice were sacrificed for bone marrow harvesting 24 h after the second treatment. There was no significant or dose-related increase in micronuclei in treated groups, while there was a clear response in the positive control. However, in the absence of cytotoxicity at bone marrow, this study provided no evidence of target cell exposure.

The main subchronic oral toxicity study was preceded by a sub-acute oral toxicity study with the same test item and in the same strain of rats to check suitability of the doses used. The systemic toxic potential of the enzyme concentrate to rats by oral administration was assessed over a period of 13 weeks, in compliance with OECD guideline 408 (rev. 1998). Four groups of 10 Sprague–Dawley rats of each sex received the test material by gavage (10 mL/kg body weight per day) for 90 days at doses of 0, 100, 500 or 1,000 mg/kg body per day. Two more groups received the test material at doses of 0 or 1,000 mg/kg body weight per day for 90 days and then were allowed to recover for a month. The control group received the vehicle (purified water). No animals died during the study and there were no differences in the recorded clinical observations between control and treated groups. There were no significant effects of treatment on feed consumption or body weight gain although the body weight of the highest dose group of both sexes was slightly lower than controls by the end of the study and this difference remained after the reversal period. No changes due to treatment were found in the ophthalmological examinations, sensory reactivity, motor activity or in the grip strength of the rats. The haematological results showed lower RBC and platelet counts compared with controls in high-dose male rats at the end of the study and higher platelet counts after the reversal period. Lower platelet counts were also present in the intermediate dose group at the end of the study. A similar lower value for platelet counts in was also seen in females at the intermediate and high doses. After the reversal period, the only difference present in high-dose females was higher white blood cell (WBC) counts. The results of the blood chemistry analyses showed higher serum alkaline phosphatase levels in intermediate and high-dose males and high-dose females at the end of the study. After the reversal period, higher serum protein and urea levels were found in high-dose males. For females, after the reversal period, levels of alanine aminotransferase alanine aminotransferase (ALAT) and calcium were higher and levels of potassium and chloride were lower than control values. Urinalysis revealed no differences between control and treated groups. Epididymis weights for all treated groups were higher than those of controls for all treated male groups at the end of the study. There was no similar difference and no other differences after the reversal period. High-dose females showed no dose-related differences in organ weights at the end of the study but the high-dose group had higher relative weights of kidney and heart after the recovery period. Gross and microscopic examination of tissues revealed no differences between high-dose and control groups.
3.2.2.5. *Bacillus licheniformis* DSM 27949 and *B. amyloliquefaciens* DSM 27947

Toxicological studies are not required for products obtained from microorganisms belonging to species that are considered suitable for the qualified presumption of safety (QPS) approach for safety assessment provided that the qualifications are met (EFSA, 2007; EFSA BIOHAZ Panel, 2017). The fermentation products containing the protease and the amylase are obtained from *B. licheniformis* DSM 27949 and from *B. amyloliquefaciens* DSM 27947, respectively. These two production strains belong to a species considered by EFSA to be suitable for the QPS approach. The strain *B. licheniformis* DSM 27949 was unambiguously identified and the relevant qualifications, absence of toxigenic potential and antimicrobial susceptibility were met. The identification of *B. licheniformis* DSM 27947 was not fully resolved between *licheniformis* or *subtilis*, but both species considered suitable for the QPS approach. The relevant qualifications, absence of toxigenic potential and antimicrobial susceptibility, were met. Therefore, for these two fermentation products toxicological studies are not required. The Panel considers that the fermentation products obtained from these strains raise no concerns for the safety of the consumer of food products obtained from animals fed with them.

3.2.2.6. Conclusions on safety for the consumer

The fermentation products from *B. licheniformis* DSM 27949 and *B. amyloliquefaciens* DSM 27947 do not raise concerns for consumer safety. The results obtained in the genotoxicity studies and in the subchronic oral toxicity studies for the products obtained from *T. citroviride* DSM 27790 and *A. niger* DSM 27958 do not indicate any reason for concern for consumer safety arising from the use of the product as a feed additive.

3.2.3. Safety for the user

No specific data has been provided; in the absence of such data, the FEEDAP Panel cannot conclude on the potential of the additive to be irritant to the skin and eyes or on its skin sensitising properties. Owing to the nature of the active substances, the additive should be considered a potential respiratory sensitiser, the dusting potential of the additive is considered as high and therefore the likelihood of exposure is high.

3.2.4. Safety for the environment

The active substances of the additive are proteins, and as such will be degraded/inactivated during passage through the digestive tract of animals or in the environment. Therefore, the additive raises no concerns for the environment.

3.3. Efficacy

The applicant submitted three efficacy trials in chickens for fattening and three in weaned piglets. All the studies considered a control diet and a diet supplemented with the recommended dose. In the case of the chickens the trials followed a $2 \times 2$ factorial design with two types of diet (wheat–maize–soya bean meal- or maize–soya bean meal-based diet) not supplemented or supplemented with FRA® Octazyme C Dry.

However, limitations in the analysed enzyme activities in the feed were found in all the trials. The analytical results for the feeds administered to the animals are presented in Appendix A (Tables A.2 and A.3). It is noted that the analytical methods used for mannanase, glucanase, protease and cellulase had a limit of detection (LOD)/quantification (LOQ) higher than the enzyme activity recommended ($< 200 \text{ UM}, < 4,000 \text{ BU}, < 1,500 \text{ UPR}$ and $< 300 \text{ UC}$ per kg feed, respectively). This fact does not allow checking the compliance with the intended dosages for these four enzyme activities. The analyses showed that in most of the cases, seven out of nine, the non-supplemented diets presented enzyme activities close to the ones in the supplemented diet or even higher than in the diet supplemented with the recommended dose. Consequently, the intended dosages of the enzymes were not confirmed. The exception to this fact were two control maize based diets in the studies done with chickens where the non-supplemented diets showed enzyme activities lower than the diet supplemented at the recommended dose. However, it is noted that in these two cases the supplementation of the diets resulted in values above the intended dosages and would not permit the Panel to conclude at the recommended dose. The FEEDAP Panel considers that these limitations do not permit to assess the efficacy of the product and therefore no conclusions can be drawn.
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\(^65\) and Good Manufacturing Practice.

4. Conclusions

The Panel cannot conclude on the safety and efficacy for the target species due to the limitations identified in the studies provided.

The use of FRA\(^\circ\) Octazyme C Dry as a feed additive does not give rise to concerns for the consumers of food derived from animals fed with it.

In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be irritant to the skin and eyes or on its skin sensitising properties but should be considered a potential respiratory sensitiser.

The use of FRA\(^\circ\) Octazyme C Dry as a feed additive poses no risks to the environment.

**Documentation provided to EFSA**

1) FRA\(^\circ\) Octazyme C Dry for chickens for fattening and weaned piglets. August 2014. Submitted by Framelco B.V.
2) FRA\(^\circ\) Octazyme C Dry for chickens for fattening and weaned piglets. Supplementary information. October 2015. Submitted by Framelco B.V.
3) FRA\(^\circ\) Octazyme C Dry for chickens for fattening and weaned piglets. Supplementary information. August 2016. Submitted by Framelco B.V.
4) FRA\(^\circ\) Octazyme C Dry for chickens for fattening and weaned piglets. Supplementary information. December 2016. Submitted by Framelco B.V.
5) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for FRA\(^\circ\) Octazyme C Dry.
6) Comments from Member States.

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\(^{65}\) 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.
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Abbreviations

ALAT Alanine aminotransferase
CFU colony forming unit
CV coefficient of variation
DON deoxynivalenol
DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen
EC Enzyme Commission
EURL European Union Reference Laboratory
FEEDAP EFSA Panel on Additives and Products or Substances used in Animal Feed
LOD limit of detection
LOQ limit of quantification
MCHC mean corpuscular haemoglobin concentration
MIC minimum inhibitory concentration
OECD Organisation for Economic Co-operation and Development
PCB polychlorinated biphenyl
PCDD polychlorinated dibenzodioxins
QPS Qualified Presumption of safety
RBC red blood cell
WBC white blood cell
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for FRA® Octazyme C Dry

In the current application, authorisation is sought under article 4 (1) for FRA Octazyme C Dry under the category/functional group 4 (a) ‘zootechnical additive’/‘digestibility enhancers’, according to the classification system of Annex 1 of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the feed additives for chickens for fattening and weaned piglets.

According to the Applicant, FRA Octazyme C Dry is a preparation containing eight enzymes with a guaranteed minimum activity of 160,000 BXU/g for endo-1,4-β-xylanase; 20,000 BU/g for endo-1,3(4)-β-glucanase; 3,200 UG/g for endo-1,4-β-glucanase; 1,000 UM/g for mannan-endo-1,4-β-mannosidase; 2,100 UP/g for pectinase; 80 GALU/g for α-galactosidase; 1,500 UPR/g for protease and 10,000 UA/g for α-amylase. The Applicant expressed the enzyme activities in different units defined as follows:

- one unit of endo-1,4-β-xylanase activity (BXU) is the amount of enzyme, which liberates one nanomole per second of reducing sugars, expressed as xylose equivalents, from the beechwood xylan substrate at pH 5.3 and 50°C;
- one unit of endo-1,3(4)-β-glucanase activity (BU) is the amount of enzyme, which liberates one nanomole per second of reducing sugars, expressed as glucose equivalents, from the barley beta-glucan substrate at pH 4.8 and 50°C;
- one unit of endo-1,4-β-glucanase activity (UG) is the amount of enzyme which catalyses the production of one micromole per minute of reducing sugars, expressed as glucose equivalents, from the carboxymethylcellulose substrate at pH 5.5 and 40°C;
- one unit of mannan-endo-1,4-β-mannosidase activity (UM) is the amount of enzyme that produces reducing sugars having a reducing power corresponding to one micromole of mannose per minute from galactomannan substrate at pH 7.0 and 50°C;
- one unit of pectinase activity (UP) is the amount of enzyme, which liberates one micromole per minute of reducing sugars, expressed as glucose equivalents, from the orange polygalacturonic acid substrate at pH 4.5 and 40°C;
- one unit of α-galactosidase activity (GALU) is defined as the amount of enzyme which degrades one micromole per minute of p-nitrophenyl α-D-galactopyranoside at pH 5.5 and 37°C;
- one unit of protease activity (UPR) is defined as the amount of enzyme which liberates one microgram per minute of phenolic compound, expressed as tyrosine equivalents from the casein substrate at pH 7.5 and 50°C; and
- one unit of α-amylase activity (UA) is the amount of enzyme which catalyses the production of one micromole per minute of reducing sugars, expressed as glucose equivalents, from the wheat starch substrate at pH 5.5 and 40°C.

FRA Octazyme C Dry is intended to be incorporated directly in feedingstuffs for chickens and weaned piglets with the following proposed minimum enzyme activities in feedingstuffs: 8,000 BXU/kg for endo-1,4-β-xylanase; 1,000 BU/kg for endo-1,3(4)-β-glucanase; 160 UG/kg for endo-1,4-β-glucanase; 50 UM/kg for mannan-endo-1,4-β-mannosidase; 105 UP/kg for pectinase; 4 GALU/kg for α-galactosidase; 75 UPR/kg for protease and 500 UA/kg for α-amylase.

The Applicant submitted eight single-laboratory validated and further verified colorimetric methods for the quantification of the active substances in the feed additive and feedingstuffs, based on the enzymatic hydrolysis:

- of xylanase on the beechwood xylan substrate at pH 5.3 and 50°C for the determination of endo-1,4-β-xylanase;
- of glucanase on the barley beta-glucan substrate at pH 4.8 and 50°C for the determination of endo-1,3(4)-β-glucanase;
- of cellulase on the carboxymethylcellulose substrate at pH 5.5 and 40°C for the determination of endo-1,4-β-glucanase;
- of mannanase on the locust bean gum substrate at pH 7.0 and 50°C for the determination of mannan-endo-1,4-β-mannosidase;
- of pectinase on the orange polygalacturonic acid substrate at pH 4.5 and 40°C for the determination of pectinase;
of α-galactosidase on the p-nitrophenyl α-D-galactopyranoside substrate at pH 5.5 and 37°C for the determination of α-galactosidase;

• of protease on casein substrate at pH 7.5 and 50°C for the determination of protease; or

• of amylase on the wheat starch substrate at pH 5.5 and 40°C for the determination of α-amylase.

Based on the performance characteristics available, the EURL recommends for official control the proposed single-laboratory validated and further verified colorimetric methods for the quantification of the eight enzymes in the feed additive.

Due to the lack of suitable experimental data, the EURL cannot evaluate the proposed method for the quantification of protease in feedingstuffs. Furthermore, the EURL considers the proposed single-laboratory validated and further verified colorimetric methods suitable (i) for the quantification of endo-1,4-β-xylanase and manan-endo-1,4-β-mannosidase at the minimum activities proposed by the Applicant and (ii) for the quantification of endo-1,3(4)-β-glucanase, endo-1,4-β-glucanase, pectinase, α-galactosidase, and α-amylase at activity levels above their respective limits of quantification (LOQ) in 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.
Appendix A – Enzyme activity in the feeds used in the tolerance and efficacy trials in target species

In the tables below, it is shown the analysed enzyme activity in the feeds used in the tolerance and efficacy trials.

Table A.1: Mean analysed enzyme activity in the diets used in the efficacy trials in chickens for fattening and piglets

| Trial    | Species    | Diet  | Xylanase BXU/kg | Mannanase Iₘ/kg | Amylase Iₐ/kg | Glucanase BU/kg | Protease Uₚ/kg | Cellulase Iₚ/kg | Pectinase Uₚ/kg | Galactosidase GALU/kg |
|----------|------------|-------|----------------|-----------------|---------------|----------------|----------------|-----------------|-------------------|-----------------------|
|          |            |       | Expected at 50 mg/kg |                 |               |                |                |                 |                   |                       |
| Tolerance| Chickens   | Control | 5,877            | 110             | 11,027        | 470            | < 10           | < 10            | 61                | 70                    |
|          |            | 50 mg/Kg | 10,830           | 139             | 9,603         | 2,640          | 151            | < 10            | 133               | 64                    |
|          |            | 5,000 mg/kg | 359,600       | 279             | 21,367        | 42,200         | 103            | 1,048           | 2,983             | 113                   |
| Tolerance| Weaned piglets (a) | Control | 122,000        | < 10            | 104,000       | 64,100         | 336            | 1,680           | 65,700            | 726                   |
|          |            | 50 mg/Kg | 20,464          | 444             | 36,300        | 3,550          | 171            | < 10            | 925               | 268                   |
|          |            | 5,000 mg/kg | 759,000       | 638             | 85,650        | 67,950         | 400            | 1670            | 51,400            | 682                   |

(a): Two basal diets, prestarter and starter, were used in the study. For the control diet, the enzyme activities provided for the starter diet were not considered since it was indicated that they belonged to the starter diet containing 5,000 mg additive/kg feed.
Table A.2: Mean analysed enzyme activity in the diets used in the efficacy trials in chickens for fattening

| Trial   | Basal diet | Diet     | Xylanase BXU/kg | Mannanase I_M/kg | Amylase I_A/kg | Glucanase BU/kg | Protease U_PR/kg | Cellulase I_C/kg | Pectinase U_P/kg | Galactosidase GALU/kg |
|---------|------------|----------|----------------|------------------|----------------|----------------|------------------|------------------|------------------|----------------------|
|         |            | Expected at 50 mg/kg | 8,000 | 50 | 500 | 1,000 | 75 | 160 | 105 | 4 |
| Efficacy 1 | Wheat | Control | 7,166 | < 200 | 15,666 | < 4,000 | < 1,500 | 320 | 49 | 264 |
|          |          | 50 mg/Kg | 7,976 | < 200 | 52,033 | 6,066 | < 1,500 | 365 | 118 | 264 |
|          | Maize    | Control | 4,433 | < 200 | 2,317 | < 4,000 | < 1,500 | < 300 | 22 | 65 |
|          |          | 50 mg/Kg | 6,223 | < 200 | 7,640 | 6,500 | < 1,500 | < 300 | 66 | 67 |
| Efficacy 2 | Wheat | Control | 7,267 | < 200 | 26,333 | 7,000 | 3,000 | 340 | 37 | 180 |
|          |          | 50 mg/Kg | 6,677 | < 200 | 24,067 | 7,050 | < 1,500 | 310 | 92 | 122 |
|          | Maize    | Control | 6,600 | < 200 | 8,333 | < 4,000 | < 1,500 | < 300 | 19 | 60 |
|          |          | 50 mg/Kg | 22,213 | < 200 | 311,077 | 58,500 | < 1,500 | 154,000 | 152 | 39 |
| Efficacy 3 | Wheat | Control | 7,900 | < 200 | 21,000 | 4,233 | 5,167 | 383 | 70 | 404 |
|          |          | 50 mg/Kg | 5,443 | < 200 | 23,533 | 6,733 | < 1,500 | 330 | 75 | 159 |
|          | Maize    | Control | 9,500 | < 200 | 10,067 | 7,867 | < 1,500 | 320 | 53 | 178 |
|          |          | 50 mg/Kg | 7,560 | < 200 | 7,073 | 5,033 | < 1,500 | < 300 | 84 | 68 |
Table A.3: Mean analysed enzyme activity in the diets used in the efficacy trials in weaned piglets

| Trial     | Diet                      | Xylanase BXU/kg | Mannanase IU/kg | Amylase IU/kg | Glucanase BU/kg | Protease UPR/kg | Cellulose UC/kg | Pectinase UP/kg | Galactosidase GALU/kg |
|-----------|---------------------------|-----------------|-----------------|--------------|----------------|-----------------|----------------|-----------------|----------------------|
| Expected at 50 mg/kg | 8,000 | 50 | 500 | 1,000 | 75 | 160 | 105 | 4 |
| Efficacy 1 | Control | 5,550 | < 200 | 2,150 | < 4,000 | < 1,500 | < 300 | 34 | 31 |
| 50 mg/Kg  | 4,705 | < 200 | 6,090 | 5,250 | < 1,500 | < 300 | 129 | 58 |
| Efficacy 2<sup>(a)</sup> | Control | 122,000 | < 10 | 104,000 | 64,100 | 336 | 1,680 | 65,700 | 726 |
| 50 mg/Kg  | 20,464 | 444 | 36,300 | 3,550 | 171 | < 10 | 925 | 268 |
| Efficacy 3 | Control | 12,550 | < 200 | 9,735 | 5,840 | < 150 | 232 | 135 | 61 |
| 50 mg/Kg  | 12,700 | < 200 | 2,135 | 5,840 | < 200 | 215 | 523 | 31 |

(a): Two basal diets, prestarter and starter, were used in the study. For the control diet, the enzyme activities provided for the starter diet were not considered since it was indicated that they belonged to the starter diet containing 5,000 mg additive/kg feed (see tolerance trial).