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Safety and efficacy of Capsozyme SB Plus (α-galactosidase and endo-1,4-β-xylanase) as a feed additive for poultry species for fattening or reared for laying and ornamental birds

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Abstract

Following a request from the European Commission, the EFSA 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 Capsozyme SB Plus as a zootechnical feed additive (digestibility enhancers) for poultry species and ornamental birds. The additive contains two enzyme activities (α-galactosidase and endo-1,4-β-xylanase) and it is presented in solid form. The α-galactosidase is produced by a non-genetically modified strain of *Aspergillus tubingensis* and the xylanase is produced by a non-genetically modified strain of *Trichoderma longibrachiatum.* Based on the results obtained in a tolerance trial performed in chickens for fattening, the FEEDAP Panel concluded that the additive is safe for the target species at the recommended levels. The mixture of the two enzyme concentrates that are used to formulate the additive, did not show any potential for a genotoxic effect in a bacterial reverse mutation assay and an *in vitro* mammalian cell micronucleus test. However, owing to the limitations identified in the subchronic oral toxicity study, the Panel could not conclude on the toxicological potential of the additive. No studies were submitted by the applicant to address the safety aspects related to the user. Therefore, the Panel could not conclude on the skin or eye irritation potential of the additive nor on its skin sensitisation potential. Owing to the proteinaceous nature of the additive it should be regarded as a potential respiratory sensitiser, but the exposure is presumed to be limited due to the low dusting potential. The Panel considered that the product, used as feed additive, poses no risks to the environment and no further environmental risk assessment is required. The Panel considered a total of five efficacy trials, however, the limited evidence of the efficacy provided by those studies did not allow the Panel to conclude on the efficacy.

Keywords: Zootechnical additive, digestibility enhancers, galactosidase, xylanase, safety, efficacy, avian species

Requestor: European Commission

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Industrial Técnica Pecuaria S.A.[2] for the authorisation of the product Capsozyme SB Plus (α-galactosidase and endo-1,4-β-xylanase), when used as a feed additive for chickens for fattening or reared for laying, other poultry species (for fattening or reared for laying) and ornamental birds (category: zootechnical additives; functional group: digestibility enhancers).[3]

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 15 May 2018.

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 Capsozyme SB Plus (enso-1,4-β-xylanase and α-galactosidase), when used under the proposed conditions of use (see Section 3.1.5).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[4] in support of the authorisation request for the use of Capsozyme SB Plus as a feed additive.

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 Capsozyme SB Plus is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a,b,c), 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, 2008), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012a,b,c) and the Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012a,b,c).

[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] Industrial Técnica Pecuaria S.A., Avinguda de Roma 7th Floor, 08011, Barcelona, Spain.
[3] During the course of the evaluation the applicant informed Commission on the will to modify the target species from those initially notified that included chickens for fattening, chickens reared for laying and minor poultry species for fattening and reared for laying.
[4] FEED dossier reference: FAD-2017-0067.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2017-0067_capsozymesbplus.pdf
[6] 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.

3. Assessment

This assessment deals with the safety and efficacy of Capsozyme SB Plus (α-galactosidase and endo-1,4-β-xylanase) as a zootechnical additive (functional group: digestibility enhancers) for chickens for fattening or reared for laying other poultry species and ornamental birds.

3.1. Characterisation

3.1.1. Characterisation of the active substances

The α-galactosidase (Enzyme Commission number, EC, 3.2.1.22; galactosidase) present in the additive is produced by a non-genetically modified strain of *Aspergillus tubingensis* originally isolated from beetroot samples, subjected to a further selection process by exposure to chemical/physical challenges. It has been deposited at the American Type Culture Collection as *Aspergillus niger* with the accession number ATCC SD 6740.[7] The identification of the production strain as *A. tubingensis* was done [redacted][8] *A. tubingensis* is considered synonymy of *A. niger* var. *tubingensis.*[9]

The endo-1,4-β-xylanase (EC 3.2.1.8; xylanase) present in the additive is produced by a non-genetically modified strain of *Trichoderma longibrachiatum*, originally isolated from soil samples and subjected to a further selection process by exposure to chemical/physical challenges. It has been deposited at the Centraalbureau voor Schimmelcultures (CBS) with the accession number CBS 139997.[10] The identification of the strain as *T. longibrachiatum* was done [redacted][11]

3.1.2. Manufacturing process

The enzymes are obtained in two separated fermentation processes. [redacted][12]

3.1.3. Characterisation of the additive

Capsozyme SB Plus is a solid product that contains the fermentation products (2% of each of the two enzymes), and kieselgur (95%)[13] and dextrin (1%) as carriers. The additive ensures a minimum enzyme activity of 40 GALU[14] (galactosidase units) and 50 AXC[15] (xylanase units) per gram of product. The batch to batch analysis in six batches was provided and showed an average of 45 GALU per gram (ranging from 42 to 50 GALU per gram; coefficient of variation, CV, 6%) and 58 AXC (ranging from 51 to 63 AXC per gram; CV 8%) per gram.[16]

Three batches were analysed for microbial contamination[17] and chemical impurities,[18] including total Enterobacteriaceae (< 30 CFU/g), *Escherichia coli* (not detected in 1 g), *Salmonella* spp. (absent in 25 g), arsenic (< 1 mg/kg), cadmium (0.55 to 0.58 mg/kg), lead (< 1 mg/kg), mercury (0.1 mg/kg), aflatoxin B1 (< 1 μg/kg), ochratoxin A (< 2 μg/kg), deoxynivalenol (< 50 μg/kg) and zearalenone (< 10 μg/kg).

[7] Technical dossier/Section II/Annex 2.2.1.2.a/Supplementary information June 2019/Annex 1/Supplementary information November 2019/Annex 1.
[8] Technical dossier/Section II/Annex 2.2.1.2.b.
[9] http://www.mycobank.org
[10] Technical dossier/Section II/Annex 2.2.1.2.c/Supplementary information June 2019/Annex 1.
[11] Technical dosier/Section II/Annex 2.2.1.2.d/Supplementary information November 2019/Annex 3.
[12] Technical dossier/Section II/Supplementary information June 2019/Annex 4 and Supplementary information November 2019.
[13] Substance under re-evaluation.
[14] one unit of *α-galactosidase* activity (GALU) is defined as the amount of enzyme which degrades one micromole per minute of para-nitrophenyl-α-D-galactopyranoside at pH 5.5 and 37°C.
[15] one unit of *endo-1,4-β-xylanase* activity (AXC) is the amount of enzyme, which liberates 0.058 micromoles per minute of reducing sugars, expressed as xylose equivalents, from a wheat arabinoxylan substrate at pH 4.7 and 30°C.
[16] Technical dossier/Section II/Annex 2.1.3.a.
[17] Technical dossier/Section II/Annex 2.1.4.a.
[18] Technical dossier/Section II/Annexes 2.1.4.b and c.

Three batches of the additive were solvent extracted and the extracts were injected to liquid Chromatography with tandem mass spectrometry (LC–MS/MS) system for further analysis of mycotoxins and other secondary metabolites.[19] The investigation included several secondary metabolites. The most relevant for the current assessment were naphtopyrones, nigragillin, pyranonigrin and ochratoxin A which may be produced by *A. tubingensis*. These compounds were not detected (ochratoxin A below 1.2 μg/kg additive; pyranonigrin 56 μg/kg; naphtopyrones and nigragillin, the limits of detection cannot be established due to the lack of standards). The presence of peptaibols, which may be produced by *T. longibrachiatum* was not studied, but the samples showed contents above the limit of detection for brevianamide F (8 μg/kg additive), tryptophol (9 μg/kg additive) and two other cyclic dipeptides (100 μg/kg additive).

The applicant provided data on the presence of antimicrobial activity in the concentrates of each enzyme which are used to produce the additive.[20] The analysis was done following the agar well diffusion method. The strains *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 and *Bacillus subtilis* ATCC 6633, were used. Tetracycline was used as positive control and water or methanol were used as negative controls. No inhibition was found around the wells with the concentrates of the enzymes (samples diluted 10 times, amount per well was 100 μL).

Regarding the presence of viable cells of the production strains in the product the applicant provided a set of data that was not further considered due to the fact that the amount of sample tested and the time of incubation were not sufficient.[21] In a second data set, the presence of the production strains was tested in three batches of the enzyme concentrate. Each batch was analysed in triplicate for each production strain.[22] In each test, a 10 g sample of each batch was diluted in 90 mL of a 0.9% NaCl solution. A total volume of 10 mL of the suspensions were plated (0.3 mL in each plate) on potato dextrose agar and incubated at 25°C for 7 days. A positive control was prepared by spiking samples of the additive treated in the same way as the test items with 1 mL of cell suspension of the production strain in order to reach 2.3×10^2 CFU/mL for *A. tubingensis* and 7×10^2 CFU/mL for *T. longibrachiatum*. A negative control was also considered (0.9% NaCl solution). The controls performed as expected. In the test samples, a total of two colonies grew in the galactosidase product, and other three colonies grew in the xylanase product. These colonies were subcultured. The growth of the subcultured colonies resembled the growth of bacteria, not of fungi, and therefore no PCR was done to check the identity.

The particle size of the product was measured in three batches of the additive by laser diffraction and showed that 42% of the particles have a size < 100 μm, less than a 10% of particles have a particle size < 10 μm.[23] Three batches were analysed for dusting potential (Stauber–Heubach method) and showed that the dusting potential is negligible (< 0.1 g/m^3).[24] The additive has a density of 575–600 kg/m^3.[25]

3.1.4. Stability and homogeneity

The shelf-life of the additive was studied in three batches of the additive that were kept at room temperature or at 40°C in plastic bags for up to 12 months.[26] The losses of enzyme activity in the samples stored at room temperature were of 7% for the xylanase and of 9% for the galactosidase, and showed reductions of 12% for the xylanase and 14% for the galactosidase when stored at 40°C.

The stability of the enzymes of the additive (one batch) in a complete (vitamin/mineral) premixture without choline chloride was studied when stored in plastic bags at 25°C for up to 6 months.[27] The results showed a decrease of 10% in the xylanase and 3% in the galactosidase enzyme activities.

The stability of one batch of the additive was assessed in feed for chickens for fattening (basal diet consisting of maize and soybean meal) in mash and pelleted form was studied when stored in plastic bags at room temperature and protected from light.[28] The effect of pelleting on the enzyme activities

[19] Technical dossier/Section II/Annex 2.2.2.2./Supplementary information June 2019/Annex 2.
[20] Technical dossier/Supplementary information November 2019/Annex 4a and 4b.
[21] Technical dossier/Supplementary information June 2019/annex 3a and 3b.
[22] Technical dossier/Supplementary information November 2019/Annex 5.a and 5.b.
[23] Technical dossier/Section II/Annexes 2.1.5 a to c.
[24] Technical dossier/Section II/Annex 2.1.5.d
[25] Technical dossier/Section II/Annex 2.1.5d.
[26] Technical dossier/Section II/Annex 2.4.1a and 2.4.1b.
[27] Technical dossier/Section II/Annex 2.4.1c and 2.4.1f for premix composition and supplementary information June 2019/ Answers to Sin 310718, answer to question 4.
[28] Technical dossier/Section II/Annexes 2.4.1d, 2.4.1e and 2.4.1g.

was also studied in the feed (temperature of the heat treatment was 70°C), and showed recoveries of the initial enzyme activity of 92% and 89% for galactosidase and xylanase, respectively. As regards the mash feed, the observed losses amounted to 3% for the xylanase and 8% for the galactosidase enzyme activities. When considering the pelleted feed, the observed losses were of 12% for the xylanase and 8% for the galactosidase activities.

The capacity of the two enzymes to homogeneously distribute was tested in feed for pigs for fattening, which can be representative of a feed for poultry.[29] Ten subsamples were analysed and the coefficient of variation of their respective enzyme activities was calculated, giving values of 6% and 8% for the xylanase and the galactosidase, respectively.

3.1.5. Conditions of use

The additive is proposed to be used as a zootechnical additive (functional group: digestibility enhancers) in feed for chickens for fattening or reared for laying, minor poultry species (for fattening or reared for laying) and ornamental birds at a minimum level of 14 GALU and 18 AXC per kg feed and at a maximum of 20 GALU and 25 AXC per kg feed.[30]

3.2. Safety

3.2.1. Safety for the target species

The applicant submitted two tolerance trials in chickens for fattening, one of which was not considered further in the assessment due to the low performance of the chickens during the study (27% below the performance objectives).[31]

In the second tolerance study, a total of 1,140 one-day-old male chickens (Ross 308) were allocated to pens in groups of 20 birds each and distributed to three dietary treatments (nineteen replicate pens per treatment).[32] Three basal diets, starter, grower and finisher based on wheat, barley, rye and soybean meal were prepared and were either not supplemented (control) or supplemented with the enzymes to provide 14/18 (minimum recommended level) or 2,000/2,500 (100-fold the maximum recommended level) GALU/AXC per kg of feed (enzyme activities were confirmed by analysis). The diets were pelleted and offered to the birds for 36 days. Body weight was measured on pen basis on days 10, 21 and 36. Feed intake was measured throughout the study period and feed to gain ratio was calculated. Mortality was recorded throughout the study. An analysis of variance (ANOVA) was done with the data obtained and group means were compared with the Tukey's test. Differences were considered significant at a level of at least $p < 0.05$.

Mortality was low (up to 2.6%) and not different between the treatments. Feed consumption was 114, 112 and 111 g for control group, the group treated with the minimum recommended level and the group treated with 100-fold the maximum recommended level, respectively. The corresponding figures for final body weight were 2.7, 2.7 and 2.8 kg and for feed to gain ratio were 1.56, 1.52 and 1.47, respectively. The birds that received the additive at the minimum recommended level showed a significantly better feed to gain ratio compared to the control and those in the group with 100-fold maximum level a better feed to gain and higher body weight compared to control.

The Panel notes that the maximum recommended dose was not tested in the study, but this would not represent a limitation for the conclusions of the study since the levels recommended are very similar. The results of the study showed no adverse effects when birds were fed with 100-fold the maximum recommended level. Therefore, the FEEDAP Panel concludes that the additive is safe at the recommended levels for chickens for fattening and the conclusion is extended to chickens reared for laying. The conclusion is also extrapolated to other poultry species (for fattening or reared for laying) and to ornamental birds.

3.2.2. Safety for the consumer

The additive contains two enzymes which are produced in two separate fermentations and the resulting products are mixed to produce the additive. The applicant provided a set of genotoxicity studies and a 90-day repeated dose oral toxicity study. The test item in these tests was the mixture of

[29] Technical dossier/Section II/Annex 2.4.2.
[30] Technical dossier/Supplementary information June 2019.
[31] Technical dossier/Section III/Annex 3.1.1.a to 3.1.1i.
[32] Technical dossier/Supplementary information June 2019/Annex 5.a.

the two enzymes used for the formulation of the final additive but without the carriers. The enzyme activities in the test item were 1,518 GALU/g and 1,946 AXC/g (enzyme activities in the final formulations are 40 GALU and 50 AXC per gram of product). For fermentation products it is recommended to test the individual fermentation products in order to maximise the likelihood to evidence any potential effect. However, since the enzyme activities of the test item are 40 times those in the final additive, the FEEDAP Panel considers that the test item assayed in those tests is a suitable one.

3.2.3. Bacterial reverse mutation assay

The test item was tested in a bacterial reverse mutation assay in *Salmonella* Typhimurium strains TA98, TA100, TA1535 and TA 1537 and in *E. coli* strain WP2uvrA (pKM101), in the presence and absence of metabolic activation, up to concentrations of 5,000 μg/plate, in compliance with OECD guideline 471.[33] The test item did not induce any increase in the number of revertants in any of the experimental conditions tested. The positive control performed as expected, demonstrating the sensitivity of the assay.

3.2.4. *In vitro* mammalian cell micronucleus test

The potential of the test item to induce micronuclei was evaluated using cultured human peripheral lymphocytes. The cells were stimulated to divide by addition of phytohaemagglutinin (PHA) 48 h before the treatment and exposed to the test item at the concentrations of 500, 1,000 and 2,000 μg/mL, in compliance with OECD guideline 487.[34] Three independent experiments were performed: 3-h exposure in the presence and absence of metabolic activation and 24-h exposure only in the absence of metabolic activation. Cytochalasin B was used to block cytokinesis, cells were harvested 24 h after the beginning of each treatment and the frequencies of micronuclei were analysed from 2,000 binucleated cells per concentration. There was no evidence of induction of micronuclei in any experimental condition while the positive controls produced a statistically significant increase in micronuclei.

3.2.5. Subchronic oral toxicity study

Sprague–Dawley rats received diets containing the test substance at 0, 1.2, 6.0 or 12.0 g/kg feed for 90 days (20 animals per treatment, 10 males and 10 females).[35,36] The study was mostly conducted in compliance with good laboratory practice (GLP) although haematological data and analytical confirmation of test diets were not generated in a compliant laboratory. The enzyme content of test diets was confirmed in one unique batch of feed used for the whole study and prepared at the beginning of the experiment. Rats were individually housed and received the diet *ad libitum*. The study was broadly in compliance with OECD guideline 408 (1998) but did not include the functional observation battery. All animals were examined daily, detailed clinical observations of all animals including ophthalmological examination was carried out weekly as well as the recording of food intake and body weight. Blood was taken from all rats for clinical chemistry and haematological examinations at the end of the study. All animals were subject to a detailed necropsy examination. A selection of organs was weighed, and a full range of organs and tissues preserved for microscopic examination. Only the samples from control and the highest dose animals were processed and examined microscopically.

There were no effects of treatment on body weight, feed intake, ophthalmoscopy or general clinical observations. Results of haematological and clinical chemistry examinations showed no differences between the treated and control groups. Relative brain weight in the lowest and highest dose females was significantly lower compared with controls. A similar difference was not seen in males. Gross and histopathological examination of organs/tissues (including brain) did not reveal any differences between treated and control groups. However, the reduction in the relative brain weight in lowest and highest dose females compared to the control cannot be assessed because of the lack of functional observation battery during the study. Therefore, the FEEDAP Panel cannot conclude on the toxicological potential of the test item.

[33] Technical dossier/Section III/Annex 3.2.2.2.b.
[34] Technical dossier/Section III/Annex 3.2.2.2.d.
[35] Technical dossier/Section III/Annexes 3.2.2.2.e to 3.2.2.2.n and Supplementary information June 2019/Annex 6.
[36] The analysis of test diets showed levels of α-galactosidase to be 1,896, 9,902 and 20,458 U/kg feed, respectively, for low intermediate and high dose diets. Comparable figures for endo-xylanase were 2,482, 12,046 and 27,170 U/kg feed.

3.2.5.1. Conclusions on safety for the consumer

The mixture of the two enzyme concentrates that are used to formulate the additive, 40 times more concentrated than the final formulations, did not show any potential for a genotoxic effect in a bacterial reverse mutation assay and an *in vitro* mammalian cell micronucleus test. However, owing to the limitations identified in the sub-chronic oral toxicity study, the Panel cannot conclude on the toxicological potential of the additive, and therefore, on the safety of the additive for the consumer.

3.2.6. Safety for the user

No studies were submitted by the applicant. Therefore, the Panel cannot conclude on the skin or eye irritation potential of the additive nor on its skin sensitisation potential. Owing to the proteinaceous nature of the additive it should be regarded as a respiratory sensitiser, but the exposure is presumed to be limited.

3.2.7. Safety for the environment

The active substances of the additive are proteins and as such will be degraded/inactivated during the passage through the digestive tract of animals. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

3.3. Efficacy

3.3.1. Efficacy for chickens for fattening

The applicant submitted studies in chickens for fattening, to support the efficacy of the additive in the target species.

A total of six studies and one study that pooled data from four of these studies were provided by the applicant. One of the trials was not considered further in the assessment due to the high mortality registered (mean value 8.8%).[37] Since the study that pooled data[38] from four studies contained the trial with the high mortality, the FEEDAP Panel did not further consider the data from this pooled analysis.

The summary of the study design of the five trials considered in the assessment is presented in Table 1 and the results of the studies are presented in Table 2. Trials 1–3 were conducted in the same trial site independently and trial 4 (tolerance trial presented in Section 3.2.1) and trial 5 were conducted in the same trial site. The information provided for the latter two does not allow to confirm whether the studies were conducted independently or not.

In all trials 1-day-old birds were used, females were used in the first three trials and males in the last two. In all studies, the animals were fed either a non-supplemented basal diet (control) or the basal diet containing the additive at the minimum recommended level. In trials 1–3, the maximum level was also considered. The intended enzyme activities were confirmed by analysis (see Table 1). The diets were administered from day 1 of life and for 35 days (trials 1–3) or 36 days (trials 4 and 5). The health and mortality were monitored throughout the study and the body weight and feed intake were recorded. Feed to gain ratio was calculated. The data was analysed with an ANOVA, using the pen as the experimental unit. Group means were compared with a *t*-test in trials 1–3 and with Tukey's in trials 4 and 5. Significance level was set at 0.05.

[37] Technical dossier/Section IV/Annex 4.3.4.a to d.
[38] Technical dossier/Section IV/Annex 4.3.5.

Table 1: Design of the studies performed with chickens for fattening

Trial	Total no birds (birds/replicate) replicates/treatment	Breed sex	Diet composition (form)	Enzyme activity (GALU/AXC per kg)	
				Intended	Analysed
1[39]	216 (6) 12	Ross 308 females	Wheat, rye, maize, soya bean (pellets)	0 14/18 20/25	0 16/16–13/17 25/30–19/24
2[40]	252 (6) 14	Ross 308 females	Wheat, rye, soya bean meal (pellets)	0 14/18 20/25	0 17/15–14/17 28/31–23/28
3[41]	252 (6) 14	Ross 308 females	Maize, soya bean meal (pellets)	0 14/18 20/25	0 13/17–14/18 16/22–18/24
4[42]	760 (20) 19	Ross 308 Male	wheat, barley, rye, soybean meal (pellets)	0 14/18	0 14/19-18/20-16/19
5[43]	760 (20) 19	Ross 308 Male	wheat, barley, rye, oats and soybean meal (pellets)	0 14/18	0 13/16-17/20-15/21

Table 2: Effects of Capsozyme SB Plus on the performance of chickens for fattening

Trial	Enzyme activity (GALU/AXC per kg)	Daily feed intake (g)	Final body weight (g)	Daily body weight gain (g)	Feed to gain ratio	Mortality (%)
1	0 14/18 20/25	92 93 93	2,112 2,143 2,180	59.1 60.0 61.1	1.32 1.29 1.26	1.4 2.8 1.4
2	0 14/18 20/25	95 95 95	2,111 2,140 2,122	59.1 60.0 59.4	1.61 1.59 1.59	1.2 1.2 0
3	0 14/18 20/25	84 81 82	1,966 1,919 1,953	54.9 53.6 54.6	1.52 1.51 1.51	3.6 2.4 1.2
4	0 14/18	114 112	2,682 2,701	73.2 73.8	1.56[a] 1.52[b]	2.4 2.2
5	0 14/18	111 112	2,647[a] 2,722[b]	72.3[a] 74.3[b]	1.54[a] 1.51[b]	1.1[a] 4.2[b]

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

No significant effects of the treatment were observed in any of the parameters studied in trials 1–3. In trial 4, a better feed to gain ratio was observed in the birds receiving the additive at the minimum recommended level compared to control. In trial 5, birds receiving the additive at the minimum recommended level showed a higher final body weight gain, better feed to gain ratio but also a higher mortality which would cast some doubts on the positive effects of the additive observed additive on the other parameters. The Panel notes that these two studies (4 and 5) may not have been performed independently and therefore the data from the two should be analysed as one unique study.

The data available in chickens for fattening does not allow the FEEDAP Panel to conclude on the efficacy of the additive in chickens for fattening. As a consequence, no conclusions can be drawn for chickens reared for laying, other poultry species (for fattening or reared for laying) and ornamental birds.

[39] Technical dossier/Section IV/Annex IV.4.3.1.a-e.
[40] Technical dossier/Section IV/Annex IV.4.3.2.a-e.
[41] Technical dossier/Section IV/Annex IV.4.3.3.a-e.
[42] Technical dossier/Supplementary information June 2019/Annex 5.
[43] Technical dossier/Supplementary information June 2019/Annex 7.

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[44] and Good Manufacturing Practice.

4. Conclusions

Capsozyme SB Plus is safe for chickens for fattening or reared for laying, other poultry species (for fattening or reared for laying) and ornamental birds at the maximum recommended level of 20 GALU and 25 AXC per kg feed.

The enzymes present in the additive did not show any potential for genotoxicity but owing to the limitations identified in the subchronic oral toxicity study, the Panel cannot conclude on the safety of the additive for consumers.

The Panel cannot conclude on the skin or eye irritation potential of the additive nor on its skin sensititisation potential due to the lack of data, however, the additive should be regarded as a respiratory sensitiser.

The use of Capsozyme SB Plus in animal nutrition raises no concerns for the environment.

The FEEDAP Panel cannot conclude on the efficacy of the additive due to the limitations of the evidence provided.

Documentation as provided to EFSA/Chronology

Date	Event
05/12/2017	Dossier received by EFSA. Capsozyme SB Plus avian species. Submitted by Industrial Tècnica Pecuaria S.A
21/12/2017	Reception mandate from the European Commission
15/05/2018	Application validated by EFSA – Start of the scientific assessment
27/06/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Methods of analysis, characterization of the additive, safety and efficacy*
14/06/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
15/08/2018	Comments received from Member States
01/04/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
27/08/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation of the additive*
30/12/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
19/03/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

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

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

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

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

[44] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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

Abbreviations

ANOVA	analysis of variance
CBS	Centraalbureau voor Schimmelcultures
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GLP	good laboratory practice
LC–MS/MS	liquid Chromatography with tandem mass spectrometry
OECD	Organisation for Economic Co-operation and Development
PHA	phytohaemagglutinin
RSDr	relative standard deviations for repeatability
RSDip	relative standard deviations for intermediate precision

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Capsozyme SB Plus

In the current application authorisation is sought under Article 4 (1) for a preparation of *alpha-galactosidase* and *endo-1,4-beta-xylanase* (*Capsozyme SB Plus)* under the category/functional group (4 a) "zootechnical additives"/"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 additive* for chickens and minor poultry species for fattening and chickens reared for laying.

According to the Applicant, *Capsozyme SB Plus* is a preparation containing the following two enzymes:

- alpha-galactosidase produced by *Aspergillus tubingensis* (ATCC SD6740) and
- endo-1,4-beta-xylanase produced by *Trichoderma longibrachiatum* (CBS 139997)

The Applicant expressed the *alpha-galactosidase* and *endo-1,4-beta-xylanase* activities in different units defined as follows:

- one unit of *alpha-galactosidase* activity (GALU) is defined as the amount of enzyme which degrades one micromole per minute of para-nitrophenyl-alpha-D-galactopyranoside at pH 5.5 and 37 °C; and
- one unit of *endo-1,4-beta-xylanase* activity (AXC) is the amount of enzyme, which liberates 0.058 micromoles per minute of reducing sugars, expressed as xylose equivalents, from a wheat arabinoxylan substrate at pH 4.7 and 30 °C.

According to the Applicant, *Capsozyme SB Plus* has a minimum enzyme activity of 40 GALU/g for *alpha-galactosidase* and of 50 AXC/g for *endo-1,4-beta-xylanase.* The product is intended to be incorporated directly in *feedingstuffs* or through *premixtures* with the following recommended enzyme activities in *feedingstuffs*: 14 GALU/kg for *alpha-galactosidase* and 18 AXC/kg for *endo-1,4-beta-xylanase*.

For the quantification of the active substances in the *feed additive, premixtures* and *feedingstuffs* the Applicant submitted different single-laboratory validated and further verified colorimetric methods, based on the enzymatic hydrolysis of the correspondent substrates by *alpha-galactosidase* and *endo-1,4-beta-xylanase*. According to the results provided by the Applicant in the frame of the respective validation and verification studies, the EURL recalculated relative standard deviations for repeatability (RSDr) and for intermediate precision (RSDip) ranging from 1.6 to 15% and from 1.5 to 18%, respectively.

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 *alpha-galactosidase* and *endo-1,4-beta-xylanase* in the *feed additive, 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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.