

Assessment on the Safety and Efficacy of a Feed Additive Consisting of a Protease (Subtilisin EC 3.4.21.62) and *Bacillus Velezensis* NRRL B-50508, *B. Velezensis* NRRL B-50509 and *B. Subtilis* NRRL B-50510 (Syncra® SWI 201 TPT) for Pigs for Fattening and Other Growing Porcine Species (Danisco (UK) Ltd.) (RP2274)

Food Standards Agency¹, Food Standards Scotland²

¹ Regulated Products Risk Assessment, Food Standards Agency, UK, ² Risk Assessment, Food Standards Scotland, UK

Keywords: Regulated Products, Feed Additives, Safety Assessment

<https://doi.org/10.46756/001c.145129>

FSA Research and Evidence

An application was submitted to the Food Standards Agency and Food Standards Scotland in October 2024 from Genencor International B.V. ("the applicant") on behalf of Danisco (UK) Ltd. for the new authorisation of an additive consisting of the subtilisin EC 3.4.21.62 (hereafter referred to as protease) produced with the genetically modified strain *Bacillus subtilis* CBS 148232, and viable spores of *Bacillus velezensis* NRRL B-50508, *B. velezensis* NRRL B-50509 and *B. subtilis* NRRL B-50510 (Syncra® SWI 201 TPT), under the category 'zootechnical additive', functional group 'digestibility enhancers' for pigs for fattening and other growing porcine species.

In 2025, the European Food Safety Authority (EFSA) published a scientific opinion on the safety and efficacy of a feed additive consisting of a protease and *Bacillus velezensis* NRRL B-50508, *B. velezensis* NRRL B-50509 and *B. subtilis* NRRL B-50510 (Syncra® SWI 201 TPT) for pigs for fattening and other growing porcine species. The FEEDAP Panel concluded that the use of a genetically modified protease production strain *B. subtilis* CBS 148232 does not raise safety concerns. Neither DNA nor viable cells of this strain were detected in the intermediate concentrates used to formulate the final additive. In addition, the use of the active agents *B. velezensis* NRRL B-50508, *B. velezensis* NRRL B-50509 and *B. subtilis* NRRL B-50510 in animal nutrition is safe for the target species, consumers and the environment. The FEEDAP Panel concluded that the additive is safe for pigs for fattening and other growing porcine species at the proposed

conditions of use and does not raise safety concerns for the environment. Furthermore, the FEEDAP Panel concluded that the additive is not irritant to the skin and eyes but should be considered a respiratory and skin sensitiser, and any exposure through respiratory tract and skin is considered a risk. When supplemented in the feed of pigs for fattening and other minor porcine species at the same physiological stage at 5,000 protease units (U)/kg and 1.5×10^8 colony forming units (CFU)/kg complete feed, Syncra® SWI 201 TPT has the potential to be efficacious.

The FSA and FSS have reviewed the applicant's authorisation application, supporting documentation, and other regulators' risk assessments, most notably the EFSA risk assessment opinion, and considers sufficient evidence has been demonstrated to conclude without the need for further questions or risk assessment.

This is a joint FSA and FSS publication.



1. Introduction

The FSA and FSS have undertaken an assessment of a feed additive submitted by Genencor International B.V. (Willem Einthovenstraat 4, Oegstgeest, Netherlands, 2342 BH) consisting of a protease produced with the genetically modified strain *Bacillus subtilis* CBS 148232, and viable spores of *Bacillus velezensis* NRRL B-50508, *B. velezensis* NRRL B-50509 and *B. subtilis* NRRL B-50510 under assimilated Regulation (EC) No 1831/2003 in each nation of Great Britain (GB). The applicant seeks a new authorisation under category 'zootechnical additive', functional group 'digestibility enhancers', for its use in feed for pigs for fattening and other growing porcine species.

In line with Article 8 of assimilated Regulation No 1831/2003, the assessment has considered and concluded whether the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by the European Food Safety Authority (EFSA) for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

To ensure the regulatory systems of the FSA/FSS are risk proportionate, and resources are used effectively, the FSA and FSS have used the evidence submitted by the applicant and other information in the public domain, including the EFSA risk assessment opinion, to provide a summary assessment of the evidence of safety presented in this report.

In 2025, EFSA published a risk assessment opinion on the safety and efficacy of a feed additive consisting of a protease and *Bacillus velezensis* NRRL B-50508, *B. velezensis* NRRL B-50509 and *B. subtilis* NRRL B-50510 (Syncra® SWI 201 TPT) for pigs for fattening and other growing porcine species (EFSA FEEDAP Panel, 2025). This opinion has been reviewed by FSA/FSS risk assessors. It has been verified that the standard approach, when compared to the relevant guidance applied in GB, has been followed and the conclusions made are consistent with the data summarised in the opinion.

The result of the assessment is that there is sufficient evidence of safety and efficacy for the UK to conclude this assessment at this time. This assessment represents the opinion of the FSA and FSS.

Table 1. Table showing products included in this assessment.

Title	Product type	Intended use/s	Intended dose/intake
Syncra® SWI 201 TPT	Feed additive	Zootechnical additive / digestibility enhancers	Minimum inclusion level of 1250 protease activity units and 3.75×10^7 total bacilli CFU per kg of complete feed.

2. Assessment

2.1. Details of other regulators' opinions

2.1.1. Current authorisation

The additive has not been authorised as feed additive in the European Union (EU) and Great Britain (GB).

2.1.2. Other regulators' opinions

In 2023, the EFSA published a scientific opinion on the safety and efficacy of a multienzyme product (AXTRA XAP) containing a protease produced with *B. subtilis* CBS 148232 (EFSA FEEDAP Panel, 2023b). The FEEDAP Panel concludes that the use of *B. subtilis* CBS 148232 as a protease production strain in the final product does not raise safety concerns as neither DNA nor viable cells of the strain are detected in the analysed samples. The additive is safe for the target species, consumers of food products obtained from animals receiving the additive, and the environment. The FEEDAP Panel could not conclude on the potential of the additive to be eye

and skin irritants or the potential to be a skin sensitiser. The additive is considered a respiratory sensitiser due to the proteinaceous nature of the active substances.

The FSA and FSS published assessment on the safety and efficacy of a feed additive consisting of endo-1,4-beta-xylanase produced by *Trichoderma reesei* ATCC PTA-5588, protease produced by *Bacillus subtilis* CBS 148232, and alpha-amylase produced by *Bacillus licheniformis* ATCC SD-6525 for all growing poultry species in 2025 (FSA and FSS, 2025). The FSA and FSS reviewed the 2023 scientific opinion published by EFSA as a part of this assessment submitted for the same additive in each nation of Great Britain (GB). The FSA and FSS reached the same conclusions regarding the safety and efficacy of the additive as EFSA in the 2023 opinion (FSA and FSS, 2025).

Following the EFSA opinion (EFSA FEEDAP Panel, 2023b), the additive consisting of endo-1,4-beta-xylanase produced by *Trichoderma reesei* ATCC PTA-5588, protease produced by *Bacillus subtilis* CBS 148232 and alpha-amylase produced by *Bacillus licheniformis* ATCC SD-6525 is authorised in the European Union (EU) for use in for chickens for fattening, chickens reared for laying and minor poultry species under Commission Implementing Regulation (EU) No 2023/1713 (EC, 2023).

2.1.3. Methodology applied in the EFSA opinion

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) assessed the safety and the efficacy of the additive, in accordance with guidance documents:

- Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017c);
- Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a);
- Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b);
- Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a);
- Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b);
- Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023a);
- Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019);

and principles in Assimilated Regulation (EC) No 429/2008.

These guidance documents were developed and implemented prior to the UK's exit from the EU and were also adopted by the FSA and FSS on exit, with exception of the Guidance on the assessment of the safety of feed additives for the user which was developed in 2023.

2.2. Section II: Identity, characterisation and condition of use

2.2.1. Characterisation of the protease production microorganism

A genetically modified strain of *B. subtilis*, deposited in the Westerdijk Fungal Biodiversity Institute with the accession number CBS 148232, is used to produce the protease present in the additive by fermentation. The identity and the characterisation of the modification of the production strain were previously assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2023b). The conclusions previously reached are still valid as no new information on the production strain has been submitted in the current application.

2.2.2. Characterisation of the active agents

The three active agents were deposited in the Agricultural Research Service Culture Collection (NRRL) and the Westerdijk Fungal Biodiversity Institute (CBS) with the accession numbers:

- NRRL B-50508/ CBS 149991 (*B. velezensis*);
- NRRL B-50509/ CBS 149981 (*B. velezensis*);
- NRRL B-50510/ CBS 149989 (*B. subtilis*).

The agents were isolated from pig manure or poultry litter and are not genetically modified according to the applicant.

Bioinformatic analysis of the whole genome sequence (WGS) data established taxonomic identification of the active agents NRRL B-50508 and NRRL B-50509 as *B. velezensis* and NRRL B-50510 as *B. subtilis*. Average genomic nucleotide identity (gANI) analysis by pairwise comparison with publicly available whole genome assembly data from four closely related species (*B. subtilis*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens* and *B. velezensis*) was used in the analysis.

In accordance with the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b), the active agents are considered to be non-toxicogenic

as no lysis of Vero cells was detected. A broth microdilution method was used to test the susceptibility of the three active agents to the recommended antibiotics in accordance with the FEEDAP Guidance (EFSA FEEDAP Panel, 2018b). The three active agents are considered susceptible to each of the relevant antibiotics as all the minimum inhibitory concentration values were below or equal to the cut-off values set for *Bacillus*, the exception of *B. subtilis* NRRL B-50510, which is resistant to one of the tested antibiotics as the cut-off value was exceeded. Presence of antimicrobial resistance (AMR) genes was interrogated by a search against the Comprehensive Antibiotic Resistance Database (CARD) and ResFinder databases with no relevant hits identified above the thresholds recommended by EFSA (EFSA, 2021). No acquired AMR gene for resistance to this tested antibiotic was found in WGS data of *B. subtilis* NRRL B-50510, and therefore previously shown resistance to one of the tested antibiotics does not raise safety concerns.

2.2.3. Manufacturing process

The manufacturing process of the protease, produced by fermentation with *B. subtilis* CBS 148232, was previously assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2020, 2023b) and was described in the current assessment (EFSA FEEDAP Panel, 2025). No antibiotics are used during the manufacturing process of the additive according to the applicant. The submitted information was considered sufficient by the FSA and FSS.

2.2.4. Characterisation of the additive

A minimum protease activity of Syncra® SWI 201 TPT is 50,000 U and a minimum total content of viable *Bacillus* spp. spores is 1.5×10^9 colony forming units (CFU) per g of additive. Analysis of 5 batches of the additive showed compliance with the predefined specifications with an average protease activity (U/g additive) of the additive of 70,340 U/g (range: 67,120–73,560 U/g) and an average total content of viable *Bacillus* spp. spores of 6.3×10^9 CFU/g additive (range: $4.6 - 8.0 \times 10^9$ CFU/g additive).

The composition of the additive (in %, w/w) is as follows: *B. velezensis* NRRL B-50508, *B. velezensis* NRRL B-50509, *B. subtilis* NRRL B-50510, protease, calcium carbonate, sodium aluminosilicate, mineral oil, sodium sulphate, talc, starch and polyvinyl alcohol (PVA).

Analysis of three batches of the additive showed following concentrations: 0.06–0.10 mg/kg of arsenic, 0.03–0.04 mg/kg of cadmium, 0.16–0.26 mg/kg of lead and < 0.005 mg/kg of mercury. Further, analysis of three batches of the additive showed levels of *Escherichia coli* not detected in 25 g, yeast and moulds < 10 CFU/g, presumptive *Bacillus cereus* group < 10 CFU/g and *Salmonella* spp. not detected in 25 g. The FEEDAP Panel concluded that the detected amounts of the impurities do not raise safety concerns.

Using *Bacillus cereus* ATCC 2, *Bacillus circulans* ATCC 4516, *E. coli* ATCC 11229, *Serratia marcescens* ATCC 14041, *Staphylococcus aureus* ATCC 6538 and *Streptococcus pyogenes* ATCC 12344 as reference strains, no antimicrobial activity was detected in the culture supernatants of the protease production strain nor in the protease concentrate used to formulate the additive under assessment.

Three batches of a filtered protease enzyme concentrate used to formulate the protease component of the additive (three samples of each batch were tested in duplicate) were investigated for the presence of viable cells and spores of the production strain. No growth was observed.

Three batches of a filtered protease enzyme concentrate used to formulate the protease component of the additive (tested in triplicate) were analysed for the presence of DNA of the production strain. No DNA of the production strain was detected.

The additive is a light granular preparation with a density of 1,280 kg/m³ at 20°C. Particle size distribution was determined in three batches by laser diffraction showing that 90% of all particles had a diameter below 750 µm, 0.9% (0.4%–1.2%) particles < 100 µm, 0.5% (0.2%–0.6%) particles < 50 µm and 0.2% (0.1%–0.3%) particles < 10 µm. The dusting potential was determined in three batches by the Stauber Heubach method, showing average of 116 mg/m³ (range: 106–133 mg/m³). The FEEDAP Panel noted that the data regarding the physical properties presented above were obtained with the additive containing protease obtained with a different production strain and with a slightly different manufacturing process that was previously assessed in 2020 (EFSA FEEDAP Panel, 2020). However, the FEEDAP Panel concluded that these changes are not expected to have a major impact on the characterisation of the additive, and therefore, the data is considered applicable to the current assessment.

On average, losses of 0.6 log in total bacilli counts and 18% in protease activity were observed after 15 months at 25°C (60% relative humidity, RH). On average, losses of 0.6 log in total bacilli counts and 38% in protease activity were observed after 12 months at 40°C (75% RH).

On average, no losses in total bacilli counts (< 0.5 log) and 9% losses in protease activity were detected in a vitamin/mineral premixture with the additive after 6 months at 25°C (60% RH). The heat treatment of mash feed supplemented with the additive (pelleted at 95°C) resulted in no losses in total bacilli counts (< 0.5 log) and 7.6% loss in protease activity. No losses in total bacilli counts (< 0.5 log) and 16% and 0% losses in protease activity were detected in mash and pelleted feed, respectively, after 3 months at 25°C (60% RH). The coefficients of variation in 10 subsamples of the mash feed were 1.7% (total bacilli counts) and 8% (protease activity).

The FSA and FSS agree with the conclusions reached for the characterisation of the additive and production strains. The amounts of the detected impurities as well as microbial contamination, do not raise safety concerns. The certificates of analysis were reviewed by the FSA and FSS and confirmed compliance with the specifications. The FSA and FSS agree that the protease obtained with a different production strain and difference in the manufacturing process are not expected to have a major impact on the characterisation of the additive, and therefore the data is considered applicable to the current assessment.

2.2.5. Conditions of use

The additive is intended for use in feed for pigs for fattening and other growing porcine species at a proposed minimum inclusion level of 1,250 protease activity units per kg and 3.75×10^7 total bacilli CFU per kg of complete feed.

The applicant requested the same conditions of use for GB as EFSA evaluated in their 2025 opinion (EFSA FEEDAP Panel, 2025). The FSA/FSS agrees with the proposed conditions of use by the applicant.

2.2.6. Conclusion on Section II: Identity, characterisation and conditions of use

The additive was fully characterised, and the identity of the active agents confirmed. The identity of the protease production strain and the characterisation of its genetic modification was described in a previous EFSA opinion (EFSA FEEDAP Panel, 2023b).

The FEEDAP Panel concluded that the amounts of the detected impurities, as well as microbial contamination, do not raise safety concerns. The information on the shelf-life and manufacturing process was provided.

FSA and FSS agree with the conclusions reached by EFSA for the characterisation of the additive, protease production strain and active agents. The FSA and FSS agree with the conditions of use proposed by the applicant.

2.3. Section III: Safety

2.3.1. Safety of the production strain and active agents

B. subtilis CBS 148232, the protease production strain, belongs to a species considered suitable for the qualified presumption of safety (QPS) approach by EFSA (EFSA BIOHAZ Panel, 2023). The safety regarding its genetic modification was evaluated by the FEEDAP Panel in the previous

assessment (EFSA FEEDAP Panel, 2023b). The FEEDAP Panel concluded that the additive does not raise any safety concerns regarding the protease production strain, as neither viable cells nor DNA of the production strain were detected in three batches of an intermediate protease enzyme concentrate used to formulate the final additive.

The active agents, spores of the species *B. velezensis* and *B. subtilis*, are also considered suitable for the QPS approach by EFSA (EFSA FEEDAP Panel, 2023b). The FEEDAP Panel concluded that the identity of the three active agents and the lack of toxigenic potential were confirmed (Section 2.2.2 Characterisation of the active agents). *B. velezensis* strains do not show resistance to antibiotics of human or veterinary importance, while *B. subtilis* NRRL B-50510 showed a low level of resistance to one of the tested antibiotics. However, no acquired resistance genes were identified in a search against the CARD and ResFinder databases. The FEEDAP Panel concluded that the three strains are presumed safe for the target species, consumer and the environment as they comply with the QPS qualifications.

The FSA and FSS agree with the conclusions reached for the safety of the production strain and active agents and agree that the production strain does not raise any safety concerns.

2.3.2. Safety for the target species

Two tolerance trials in weaned piglets were submitted by the applicant using an additive containing the three active agents and the same protease. However, the protease was produced with a different production strain. Both production strains qualify for the QPS status, and therefore no safety concerns regarding the strains were raised. The FEEDAP Panel considered the two tolerance trials relevant to the current safety assessments of the target species as the protease produced by both strains is the same.

2.3.2.1. Tolerance trials in weaned piglets

The applicant provided description and details of both tolerance trials, which was assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2025). The information was requested to be confidential. The submitted information was considered sufficient by the FSA and FSS.

The effects of the dietary supplementation in weaned piglets for the first trial are summarised in [Table 2](#). The overall mortality was not affected by additive supplementation and was considered within commercial standards with no animals being culled. No differences ($p > 0.05$) in any of the evaluated performance parameters and no significant differences in the haematological profile and serum biochemistry parameters were observed for the overall study period between groups. The only exception was the lower glucose concentration in the 40x minimum recommended

level group compared to the other groups; however, it was not considered an adverse effect. No differences were observed between groups in relative weight of the organs or pathological lesions in the gross evaluation of the necropsied animals.

Table 2. Effects of the dietary supplementation of weaned piglets with Syncra® SWI 201 TPT on selected parameters.

	Control	4x	40x
Protease activity (U/kg feed)			
Intended (pre-/starter)	0/0	5,000	50,000
Analysed (pre-/starter)	<LOD/<LOD	5,639/4,273	69,965/62,738
Total bacilli count (CFU/kg feed)			
Intended (pre-/starter)	0	1.5×10^8	1.5×10^9
Analysed (pre-/starter)	$1.2 \times 10^8/2.5 \times 10^7$	$1.7 \times 10^8/1.1 \times 10^8$	$1.6 \times 10^9/1.9 \times 10^9$
Mortality and culling (%)	2.1	3.1	1.0
Performance parameters			
Final body weight (kg)	26.6	26.5	26.3
Average daily gain (g)	429	428	423
Average daily feed intake (g)	680	637	659
Feed-to-gain ratio	1.59	1.49	1.57
Blood parameters			
Platelets ($10^3/\mu\text{L}$)	281.4 ^{ab}	329.2 ^a	273.3 ^b
Ca (mg/dL)	9.51 ^{ab}	9.69 ^a	9.29 ^b
Glucose (mg/dL)	112 ^a	119 ^a	95.7 ^b

a,b Mean values within a line with a different superscript are significantly different $p < 0.05$.

The applicant provided details of the second trial, which were assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2025). The FEEDAP Panel noted that some relevant parameters (prothrombin time, phosphate, bilirubin, acute phase protein, LDH) were not provided in accordance with the guidance for the safety of the target species (EFSA FEEDAP Panel, 2017c, 2017a, 2017b). In addition, as no specifications of the methods used for the analysis of blood parameters were provided, which raised concerns regarding the reliability of the blood data.

In the second trial, the overall mortality/culling was within commercial standards and unaffected by additive supplementation; the rates were 2.7 for the control, 0 for the 4x group and 2.7 for the 40x group. No adverse effect on zootechnical performance were observed with the supplementation of the additive up to 40x the recommended level. The average daily feed intake (0.977 kg/piglet/day), the average final body weight (30.88 kg), the average body weight gain (0.569 kg/day) and the feed-to-gain ratio (1.72) between the experimental groups showed no significant differences.

The FSA and FSS noted differences in haematological profile and serum biochemistry parameters in the first trial. However, these findings were not considered adverse as the observed values were within the historical control range in pigs.

2.3.2.2. Conclusion on the safety for the target species

At 40× the minimum recommended use level, no adverse effects were observed in weaned piglets when supplemented with the additive. The FEEDAP Panel concluded that the additive is safe for weaned piglets at 4× minimum proposed use level (5,000 U protease/kg and 1.5×10^8 CFU/kg feed). Furthermore, the FEEDAP Panel extended the conclusion to pigs for fattening and extrapolated to all growing porcine species.

The FSA and FSS agree with the conclusions reached by EFSA that the additive is safe for weaned piglets at 4× minimum proposed use level (5,000 U protease/kg and 1.5×10^8 CFU/kg feed), extension of the conclusions to pigs and extrapolated to all growing porcine species.

2.3.3. Safety for the consumer

The *B. subtilis* CBS 148232, protease production strain, and active agents *B. velezensis* NRRL B-50508, *B. velezensis* NRRL B-50509 and *B. subtilis* NRRL B-50510 qualify for the QPS approach and are presumed safe for the consumer. The FEEDAP Panel concludes that the additive, used as a feed additive for pigs for fattening and other growing porcine species, is safe for the consumers.

The FSA and FSS agrees with the conclusions reached by EFSA for the safety for the consumer as the production strain and active agents qualify for the QPS approach.

2.3.4. Safety for the user

An acute skin irritation study performed according to the OECD Guideline indicated that the additive is not irritant to the skin (UN-GHS 'No Category'). An acute eye irritation study performed according to OECD Guideline 405 showed that the additive is not irritant to the eyes (UN-GHS 'No Category'). Inhalation exposure of users is likely due to the highest analysed dusting potential of 133 mg/m³. The additive should be considered a respiratory and skin sensitiser due to its proteinaceous nature, and any exposure through the respiratory tract and skin is considered a risk.

The FSA and FSS agree with the conclusion reached by EFSA for the safety of the user. The additive is not a skin and eye irritant but should be considered a respiratory and skin sensitiser due to its proteinaceous

nature and any exposure through the respiratory tract and skin is considered a risk. User exposure by inhalation is likely due to the high dusting potential.

2.3.5. Safety for the environment

The production strain *B. subtilis* CBS 148232 and active agents *B. velezensis* NRRL B-50508, *B. velezensis* NRRL B-50509 and *B. subtilis* NRRL B-50510 are presumed safe for the environment based on the QPS approach. The FEEDAP Panel concludes that the additive is safe for the environment as neither DNA nor cells of the production strain were detected in the final product. In addition, the protease, a protein, will be degraded/inactivated during the passage through the digestive tract of animals or in the environment.

The FSA and FSS agree with the conclusion reached by EFSA for the safety of the environment. The production strain and active agents are presumed safe for the environment based on the QPS approach and no DNA or cells of the production strain were detected in the final product.

2.3.6. Conclusion on Section III: Safety

The FEEDAP Panel concludes that the use of *B. subtilis* CBS 148232 as a protease production strain and the active agents *B. velezensis* NRRL B-50508, *B. velezensis* NRRL B-50509 and *B. subtilis* NRRL B-50510 in animal nutrition is presumed safe for the target species, consumers and the environment. Neither viable cells nor DNA of the protease production strain were detected in the intermediate concentrates used to formulate the final additive. The additive is considered safe for pigs for fattening and other growing porcine species at 5,000 U protease/kg and 1.5×10^8 CFU/kg feed. The additive is safe for the consumers and the environment. The additive is not irritant to the skin or eyes but should be considered a respiratory and skin sensitiser due to its proteinaceous nature. In addition, any exposure through respiratory tract or skin is considered a risk.

The FSA and FSS agree with the conclusion reached by EFSA for the safety of the target species, consumers, user and environment.

2.4. Section IV: Efficacy as a zootechnical additive

2.4.1. Efficacy for pigs for fattening

One short-term and three long-term trials in pigs for fattening were submitted by the applicant. The data on the apparent total tract digestibility of the nutrients of the diet, including nitrogen, was provided in the short-term trial. The FEEDAP Panel concluded that data on zootechnical

performance is needed as the additive is composed of an enzyme and three active agents. The short-term study was not considered further as evidence of the efficacy as no data on the nitrogen urinary content were provided and the collection period was limited to 2 days.

The effect of the additive on the zootechnical performance of pigs for fattening was evaluated in the three long-term trials. The data were assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2025). The information was requested to be confidential. The submitted information was considered sufficient by the FSA and FSS.

2.4.2. Conclusion of the efficacy

The FEEDAP Panel concluded that the performance of pigs for fattening improved when additive was added at 1,250 U protease/kg and 3.75×10^7 CFU/kg feed in two trials and at 5,000 U protease/kg and 1.5×10^8 CFU/kg feed in the third trial. The FEEDAP Panel concluded that the additive showed potential to be efficacious when supplemented at 5,000 U protease/kg and 1.5×10^8 CFU/kg in the feed for pigs for fattening and this conclusion can be extrapolated to other minor porcine species at the same physiological stage.

The FSA and FSS agree with the conclusions reached on the efficacy, that the additive showed potential to be efficacious when supplemented at 5,000 U protease/kg and 1.5×10^8 CFU/kg in the feed for pigs for fattening, and that this conclusion can be extrapolated to other minor porcine species at the same physiological stage. This conclusion is supported by the guidance that is also applicable in GB. This demonstrates that the additive satisfies the nutritional needs of animals.

3. Analytical method evaluation

The FSA/FSS evaluated the EURL analytical method evaluation, noting it was carried out in 2019, when the UK was still part of the EU and would have participated of their approval. No concerns are raised at this stage for the validity of the methods for UK/GB use, and therefore the FSA/FSS accept the EURL analytical method evaluation report (EURL, 2019). The FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The FEEDAP Panel concluded that the use of *B. subtilis* CBS 148232 as a protease production strain and the active agents *B. velezensis* NRRL B-50508, *B. velezensis* NRRL B-50509 and *B. subtilis* NRRL B-50510 in animal nutrition is presumed safe for the target species, consumers, and the environment. Neither viable cells nor DNA of the protease production

strain were detected in the intermediate concentrates used to formulate the final additive. The additive is considered safe for pigs for fattening and other growing porcine species at 5,000 U protease/kg and 1.5×10^8 CFU/kg feed. The additive is safe for the consumers and the environment. The additive is not irritant to the skin or eyes but should be considered a respiratory and skin sensitiser due to its proteinaceous nature. In addition, any exposure through respiratory tract or skin is considered a risk.

The FEEDAP Panel concluded that the additive showed potential to be efficacious when supplemented at the maximum proposed dose of 5,000 U protease/kg and 1.5×10^8 CFU/kg in the feed for pigs for fattening, and this conclusion can be extrapolated to other minor porcine species at the same physiological stage.

5. Caveats and uncertainties

The FEEDAP Panel concluded that the additive showed potential to be efficacious when supplemented at 5,000 U protease/kg and 1.5×10^8 CFU/kg in the feed for pigs for fattening, which is 4 times the minimum recommended inclusion level in completed feed.

6. FSA/FSS conclusions for GB risk analysis

The application has been assessed in line with the applicable guidance and is partially based on considerations of detailed proprietary information available to the Panel, which were also submitted to the FSA and FSS. The EFSA opinion (EFSA FEEDAP Panel, 2025) identified and characterised the hazards present from the proposed use and concluded that there is sufficient information to enable an assessment of exposure, which is also relevant to GB. The conclusions of the EFSA opinion have been reviewed in detail by the FSA and FSS, and are considered appropriate and consistent, including the caveats and uncertainties identified in the opinion which are applicable to GB. Sufficient evidence has been demonstrated to conclude without further questions or risk assessment.

Abbreviations

Abbreviation	Definition
AMR	Antimicrobial resistance
ANI	Average nucleotide identity
BW	Body weight
CFU	Colony forming units
CV	Coefficient of variation
EU	European Union
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory

Abbreviation	Definition
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FSA	Food Standards Agency
FSS	Food Standards Scotland
GB	Great Britain
LOD	Limit of detection
OECD	Organisation for Economic Co-operation and Development
PVA	Polyvinyl alcohol
RH	Relative humidity
RP	Regulated product
U	Units
UK	United Kingdom
WGS	Whole genome sequence

Published: November 27, 2025 GMT.



This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CCBY-4.0). View this license's legal deed at <http://creativecommons.org/licenses/by/4.0> and legal code at <http://creativecommons.org/licenses/by/4.0/legalcode> for more information.

References

- EC (European Commission). (2023). *Commission Implementing Regulation No 2023/1713 of the European Parliament and of the Council on additives for use in animal nutrition*. https://eur-lex.europa.eu/eli/reg_impl/2023/1713/oj/eng
- EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (2023). Update of the list of qualified presumption of safety (QPS) recommended microorganisms intentionally added to food or feed as notified to EFSA. *EFSA Journal*, 21(1), 7747. <https://doi.org/10.2903/j.efsa.2023.7747>
- EFSA (European Food Safety Authority). (2021). EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. *EFSA Journal*, 19(7), 6506. <https://doi.org/10.2903/j.efsa.2021.6506>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2017a). Guidance on the assessment of the safety of feed additives for the consumer. *EFSA Journal*, 15(10), 5022. <https://doi.org/10.2903/j.efsa.2017.5022>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2017b). Guidance on the assessment of the safety of feed additives for the target species. *EFSA Journal*, 15(10), 5021. <https://doi.org/10.2903/j.efsa.2017.5021>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2017c). Guidance on the identity, characterisation and conditions of use of feed additives. *EFSA Journal*, 15(10), 5023. <https://doi.org/10.2903/j.efsa.2017.5023>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2018a). Guidance on the assessment of the efficacy of feed additives. *EFSA Journal*, 16(5), 5274. <https://doi.org/10.2903/j.efsa.2018.5274>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2018b). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*, 16(3), 5206. <https://doi.org/10.2903/j.efsa.2018.5206>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2019). Guidance on the assessment of the safety of feed additives for the environment. *EFSA Journal*, 17(4), 5648. <https://doi.org/10.2903/j.efsa.2019.5648>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2020). Scientific opinion on the safety and efficacy of Axtra® XAP 104 TPT (endo-1,4-xylanase, protease and alpha-amylase) as a feed additive for chickens for fattening, laying hens and minor poultry species. *EFSA Journal*, 18(6), 6165. <https://doi.org/10.2903/j.efsa.2020.6165>

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2023a). Guidance on the assessment of the safety of feed additives for the users. *EFSA Journal*, 21(12), 8469. <https://doi.org/10.2903/j.efsa.2023.8469>

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2023b). Scientific opinion on safety and efficacy of a feed additive consisting of endo-1,4-beta-xylanase produced by *Trichoderma reesei* ATCCPTA-5588, protease produced by *Bacillus subtilis* CBS148232, and alpha-amylase produced by *Bacillus licheniformis* ATCC SD-6525 (Aextra® XAP 104 TPT) for chickens for fattening, laying hens and minor poultry species (Genencor international B.V.). *EFSA Journal*, 21(2), 7816. <https://doi.org/10.2903/j.efsa.2023.7816>

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2025). Scientific opinion on safety and efficacy of a feed additive consisting of protease and *Bacillus velezensis* NRRL B-50508, *B. velezensis* NRRLB-50509 and *B. subtilis* NRRL B-50510 (Syncra® SWI 201 TPT) for pigs for fattening and other growing porcine species (Danisco(UK) Ltd.). *EFSA Journal*, 23(2), 9265. <https://doi.org/10.2903/j.efsa.2025.9265>

EURL-FA (European Reference Laboratory for Feed Additives). (2019). *CRL Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of Feed Additive according to Regulation (EC) No 1831/2003*. https://joint-research-centre.ec.europa.eu/document/download/b3104eb4-55ab-4584-954b-0660ce46bb67_en?filename=finrep_fad-2019-0044_syncra.pdf

FSA & FSS (Food Standards Agency & Food Standards Scotland). (2025). Assessment on the safety and efficacy of a feed additive consisting of endo-1,4-beta-xylanase produced by *Trichoderma reesei* ATCC PTA-5588, protease produced by *Bacillus subtilis* CBS 148232, and alpha-amylase produced by *Bacillus licheniformis* ATCC SD-6525 for all growing poultry species (Genencor International B.V.) (RP2115). *FSA Research and Evidence*. <https://science.food.gov.uk/article/129719>