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Safety and efficacy of a feed additive consisting of serine protease produced by *Bacillus licheniformis* DSM 19670 (Ronozyme® ProAct) for chickens for fattening (DSM Nutritional Products Ltd.)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Fašmon Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Pier Sandro Cocconcelli, Boet Glandorf, Lieve Herman, Miguel Prieto Maradona, Maria Saarela, Montserrat Anguita, Jaume Galobart, Orsolya Holczknecht, Paola Manini, Fabiola Pizzo, Jordi Tarrés-Call and Elisa Pettenati

Abstract

Ronozyme® ProAct is the trade name of the feed additive under assessment and contains serine protease produced by a genetically modified strain of *Bacillus licheniformis*. Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Ronozyme® ProAct when used as a zootechnical additive for chickens for fattening. The additive is available in coated thermotolerant granulated and liquid forms (Ronozyme® ProAct CT/L). The production strain and its recombinant DNA were not detected in an intermediate concentrated product used to produce the final formulations. The final products do not trigger a safety concern with regard to the genetic modification. Based on the results obtained in a tolerance study in chickens for fattening and the data from a subchronic oral toxicity study the FEEDAP Panel concluded that the additive is safe for chickens for fattening. The FEEDAP Panel concluded that the use of Ronozyme® ProAct CT/L as a feed additive gives rise to no concern for consumers and for the environment. The additive, in either form, is not an eye irritant but should be considered a skin irritant. In the absence of data, no conclusions on the skin sensitisation potential can be reached. Owing to the proteinaceous nature of the active substance it should be considered a respiratory sensitiser. The FEEDAP Panel also concluded that the additive has the potential to be efficacious at 15,000 PROT/kg compound feed for chickens for fattening.

Keywords: Zootechnical additive, digestibility enhancer, serine protease, Ronozyme® ProAct, chickens for fattening, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2019-00156

Correspondence: feedap@efsa.europa.eu

Panel members: Giovanna Azimonti, Vasileios Bampidis Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

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 DSM Nutritional Products Sp. z o.o. on behalf of DSM Nutritional Products Ltd.[2] for authorisation of the additive that contains serine protease produced by *Bacillus licheniformis* DSM 19670 (Ronozyme® ProAct CT/L), when used as a feed additive for chickens for fattening (category: zootechnical additives; functional group: digestibility enhancers).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 11 June 2019.

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 additive that contains serine protease produced by *B. licheniformis* DSM 19670 (Ronozyme® ProAct CT/L), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The additive contains serine protease which is produced by a genetically modified strain of *B. licheniformis* (DSM 19670) and its trade name in granulate and liquid forms is Ronozyme® ProAct CT/L.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) and the Panel on Genetically Modified Organisms (GMO) have issued an opinion on the safety and efficacy of this additive (including the safety of the genetic modification) for chickens for fattening (EFSA, 2009).

The additive has been previously authorised in the European Union (EU) (until 13 January 2020) as a feed additive for chickens for fattening.[3] However, as an application for renewal of Regulation (EU) No 8/2010 was not sent 1 year before its expiry date, the authorisation of the additive expired and the applicant submitted a new application for authorisation of the additive.

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 the product that contains serine protease produced by *B. licheniformis* DSM 19670 (Ronozyme® ProAct CT/L) as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the Ronozyme® ProAct (serine protease) in animal feed. The Executive Summary of the EURL report can be found in Annex A.[5]

[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] DSM Nutritional Products Ltd, Wurmisweg 576 4303 Kaiseraugst Switzerland, represented in the EU by DSM Nutritional Products Sp. z o.o., Tarczyńska 113 96-320 Mszczonów Poland.

[3] Commission Regulation (EU) No 8/2010 of 23 December 2009 concerning the authorisation of the serine protease produced by *Bacillus licheniformis* (DSM 19670) as a feed additive for chickens for fattening (holder of the authorisation DSM Nutritional Products Ltd, represented by DSM Nutritional Products Sp.Z.o.o).

[4] FEED dossier reference: FAD-2019-0010.

[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2019-0010_ronozyme_proact.pdf.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the product that contains serine protease produced by *B. licheniformis* DSM 19670 (Ronozyme® ProAct CT/L) is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a), 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 consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

This opinion assesses the safety and efficacy of the product that contains serine protease (protease (E.C.3.4.21-)) produced by *B. licheniformis* DSM 19670 (Ronozyme® ProAct CT/L) as a zootechnical additive (functional group digestibility enhancers) for chickens for fattening. It will be hereafter referred to as Ronozyme® ProAct CT/L.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The active substance is a serine protease produced by a genetically modified strain of *Bacillus licheniformis* which is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) with accession number DSM 19670.[7,8]

[redacted][9] [redacted] confirmed its identity as *B. licheniformis*.[10] [redacted]

The production strain was tested for its susceptibility to all the antimicrobials listed for *Bacillus* in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a).[11] [redacted] (EFSA FEEDAP Panel, 2018a). [redacted].

The WGS based data of the production strain was interrogated for the presence of antimicrobial resistance (AMR) genes [redacted][11,12] Therefore, no acquired AMR genes have been identified.

The toxigenic potential of *B. licheniformis* DSM 19670 was assessed according to the Guidance on the characterisation of microorganisms used as feed additives or as production organisms

[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.
[7] Technical dossier/Section II/Annex 2-8.
[8] Technical dossier/Supplementary information January 2020/Submitted/A. characterization/Appendix 1.
[9] Technical dossier/Supplementary information January 2020/Submitted/A. characterization/Appendix 2.1.1.
[10] Technical dossier/Supplementary information January 2020/Submitted/A. characterization/Appendix 2_version 2.0.
[11] Technical dossier/Supplementary information January 2020/Submitted/A. characterization/Appendix 5_version 2.0.
[12] Technical dossier/Supplementary information June 2020/Annex_2.

(EFSA FEEDAP Panel, 2018a).[13] No lysis of Vero cells was detected, so *B. licheniformis* DSM 19670 is considered to be not toxigenic.

The assessment of the genetic modification of the production strain was performed in a previous evaluation (EFSA, 2009). The applicant has provided the description of the genetic modification using the WGS-based data.[14]

3.1.1.1. Information related to the genetically modified microorganism

Characterisation of the parental microorganism

The parental strain is [redacted][15]

Description of the genetic modification

[redacted][14]

[redacted][15]

[redacted][16]

[redacted][11]

3.1.2. Manufacturing process

The production process was assessed in a previous evaluation (EFSA, 2009) and has not been modified. The serine protease is produced by [redacted] using *B. licheniformis* DSM 19670.

[redacted][17]

Annex B.

[13] Technical dossier/Supplementary information January 2020/Submitted/A. characterization/Appendix 6.
[14] Technical dossier/Supplementary information January 2020/Submitted/A. characterization/Appendix 4_version 2.0.
[15] Technical dossier/Supplementary information January 2020/Submitted/A. characterization/Appendix 3_version 2.0.
[16] Technical dossier/Section II/Appendix 2-15 and Appendix 2-16.
[17] Technical dossier/Section II/Appendix 2-7.

3.1.3. Characterisation of the additive

The additive is available in two forms, a coated thermo-tolerant granulate formulation (Ronozyme® ProAct CT) and a liquid one (Ronozyme® ProAct L).

These two formulations were fully characterised and described in a previous opinion (EFSA, 2009).

The two formulations ensure a minimum guaranteed enzyme activity of 75,000 PROT[18]/g. The batch-to-batch variation was studied in eight recent batches of each formulation.[19,20] The mean enzyme activity in Ronozyme® ProAct CT was 81,800 PROT/g (range 76,800–85,200 PROT/g) and in Ronozyme® ProAct L was 85,767 PROT/g (range 84,227–87,682 PROT/g).

Ronozyme® ProAct CT contains the serine protease (total organic solids 7.7% w/w), cellulose (9%), dextrin (5%), sucrose (3%) and sodium sulfate (61.6%) added before granulation, and calcium carbonate (7%) and palm oil (6%), which are used for coating. Water represents < 1%. Ronozyme® ProAct CT displays off-white to light brown colour with a bulk density and a tapped density of 1,000 kg/m^3 (range 990–1,020 kg/m^3) and 1,130 kg/m^3 (range 1,110–1,140 kg/m^3), respectively.[21] A 95% of the particles is in the range of 0.15 to 0.85 mm particle size. The preparation is practically dust-free, confirmed by the Heubach test (1 mg dust in 25 g).

Ronozyme® ProAct L is a transparent brown liquid preparation with a density of 1,160 kg/m^3, a pH of 4.8, a viscosity lower than 11 cP (measured at 25°C) and a surface tension of 36–39 Nm.[22] It contains the serine protease (total organic solids 7.7% w/w), water (52%) and potassium sorbate, sodium benzoate, sorbitol and glycerol as preservatives and stabilisers (40.3%).

Three batches of each formulation were analysed for chemical contamination.[20] The analysis of chemical contamination for the two formulations included arsenic (< limit of quantification (LOQ)), lead (< LOQ), cadmium (< LOQ), mercury (< LOQ) and total heavy metals (average 5.4 mg/kg (range 5.30–5.50 mg/kg) for the solid formulation and 4.10 mg/kg for all three batches of the liquid formulation).[23] Six batches for both formulations were analysed for microbial contamination and included total viable counts (≤ 100 CFU/g for the solid formulation and from < 100 to 1,400 CFU/g for the liquid formulation), coliforms (< 4 CFU/g, except in two batches of the liquid formulation which showed 12 CFU/g), *Escherichia coli* (absent in 25 g) and *Salmonella* spp. (absent in 25 g).[19,20] Three batches for both formulations were analysed for the presence of *Bacillus cereus* and all six batches showed results below the limit of detection (LOD, < 10 CFU/g).[24]

The presence of viable cells of the production strain was investigated in three batches [redacted][25] No growth was detected. [redacted] (see Section 3.1.2)

The presence of DNA from the production strain was investigated in three batches [redacted][26] The analysis showed no amplification in the samples. [redacted]

[18] One protease Unit (PROT) is the amount of enzyme that releases 1 μmol of *p*-nitroaniline from 1 mmol/L substrate (Suc-Ala-Ala-Pro-Phe-pNA) per minute at pH 9.0 and temperature 37°C.
[19] Technical dossier/Section II/Appendix 2-13.
[20] Technical dossier/Supplementary information January 2020/Submitted/A. characterization/Appendix 7.
[21] Technical dossier/Section II/Appendix 2-24. Data reported in the previous opinion (EFSA, 2009).
[22] Technical dossier/Section II/Appendix 2-25. Data reported in the previous opinion (EFSA, 2009).
[23] Technical dossier/Supplementary information October 2020/ProAct SIn answer EFSA August 2020 - Annex.pdf., reply to question 4. LOQ in mg/kg were 0.3 for arsenic, 0.5 for lead, 0.05 for cadmium and 0.05 for mercury.
[24] Technical dossier/Supplementary information October 2020/ProAct SIn answer EFSA August 2020 - Annex.pdf., reply to question 3 and Annex_5.
[25] Technical dossier/Supplementary information January 2020/Submitted/A. characterization/Appendix 8_version 3.
[26] Technical dossier/Supplementary information June 2020/Annex_1.

3.1.4. Stability and homogeneity

Shelf-life and stability of both forms of the additive, and their capacity to be uniformly distributed in premixtures and feed for the target species, were addressed in a previous opinion (EFSA, 2009). The applicant provided new data to support the shelf-life of both forms of the additive up to two years and the stability of Ronozyme® ProAct CT in premixtures up to six months.[27,28]

The shelf-life of Ronozyme® ProAct CT was evaluated in three batches when stored in closed vials for up to 12 months at different temperatures: –18, 10, 25, 35, 40, 50°C and up to 24 months at –18, 10, 25, 35°C. Recoveries were expressed as % of the activity of the sample kept at –18°C at the same time point. No losses were observed after 12 and 24 months at 10, 25 and 35°C. Recoveries were 96% and 95% for the samples kept at 40 and 50°C after 12 months, respectively. The stability of those three batches was also tested when kept for three months in an open vial at 40°C and 60% HR; the recovery was 86%.

Regarding Ronozyme® ProAct L, three batches were stored in closed vials up to 18 months at –18, 10, 25, 35, 40, 50°C and up to 24 months at –18, 10, 25, 35°C. Recoveries were 95, 95, 82, 72 and 31% for the samples kept at 10, 25, 35, 40 and 50°C after 18 months, respectively, and 97, 95 and 79% for the samples kept at 10, 25 and 35°C after 24 months, respectively.

The stability of Ronozyme® ProAct CT (three batches) in a vitamin premixture or a complete premix (containing choline chloride and trace elements) for chickens was studied when added at 30,000,000 PROT/kg and 1,500,000 PROT/kg, respectively, and stored at 25°C for up to three or six months. The recovery values were 93% after three months for the vitamin and complete premixtures, and 88% and 87% after six months for the vitamin and complete premixture, respectively.

3.1.5. Conditions of use

Ronozyme® ProAct CT/L is intended to be used in feed for chickens for fattening at the recommended level of 15,000 PROT/kg compound feed, as it was previously authorised.

3.2. Safety

3.2.1. Safety of the production organisms

The production strain *B. licheniformis* DSM 19670 was developed from [redacted] The production strain belongs to a species, *B. licheniformis*, that is suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2020). The genetic modifications performed to obtain the production strain DSM 19670 have the purpose [redacted] None of the introduced modifications raise a safety concern. The identity of the strain has been unambiguously established. Evidence was provided on the lack of toxigenic potential of the strain and on the absence of acquired AMR genes. The production strain and its DNA were not detected in a liquid concentrate representative of both final formulations. Therefore, the final products do not give raise to any safety concern with regard to the genetic modification of the production strain.

In addition, the information provided to characterise the [redacted] (see Annex B) allows the Panel to conclude that the use of [redacted] does not give raise to safety concerns.

3.2.2. Safety for the target species

In order to support the safety of the additive for the target species, the applicant submitted a tolerance study and made reference to the results obtained in a subchronic oral toxicity in rats. Both studies were already submitted with a previous application and assessed (EFSA, 2009).

The FEEDAP Panel (EFSA, 2009) evaluated in the past the tolerance trial performed with Ronozyme® ProAct CT which showed that chickens for fattening tolerated 10 times the recommended level proposed (15,000 PROT/kg feed) without adverse effects on performance or health.[29]

[27] Technical dossier/Section II/ Annex 2-22.
[28] Technical dossier/Section II/ Annex 2-23.
[29] Technical dossier/Section III/ Annex 3-1.

Moreover, the applicant provided a subchronic oral toxicity study in rats (OECD Guideline 408) which was assessed in a previous opinion (EFSA, 2009).[30] The results of the study indicate a no observed adverse effect level (NOAEL) of 287,469 PROT/kg body weight/day. From this NOAEL the maximum safe level for chickens for fattening in feed was calculated according to the Guidance on the safety for the target species (EFSA FEEDAP Panel, 2017b). The result of the calculation was 32,021 PROT/kg feed, and this would support the results of the tolerance study in which a margin of safety of 10-fold was established.

Considering that the additive has been authorised for ten years, the applicant provided also a literature search in order to support the safety of the additive for the target species. The applicant searched in a total of six relevant databases (Scopus, Medline, Web of Science Core collection, BIOSIS Citation Index, Russian science Citation Index and SciELO Citation Index).[31] The search covered the period 2008–2019, the key words were in English and the search terms and search strategy were provided. The main search terms were 'protease' and included terms relevant for target species safety and for toxicological aspects and the donor organism. The search did not identify relevant hits.

Therefore, the FEEDAP Panel concludes that Ronozyme® ProAct CT/L is safe for chickens for fattening at the recommended level of 15,000 PROT/kg feed.

3.2.3. Safety for the consumer

The enzyme is produced by a genetically modified strain of *B. licheniformis*; this species is considered to qualify for the QPS approach to safety assessment. The identity of the strain was established, and the qualifications met. None of the introduced modifications raise a safety concern. Therefore, the production strain is presumed safe and no concerns would raise for the consumer from the fermentation product obtained from this strain.

The applicant submitted a bacterial reverse mutation test,[32] an *in vitro* mammalian chromosome aberration test[33] and a subchronic oral toxicity study[30] which had been already evaluated in a previous opinion (EFSA, 2009) and are considered supportive of the safety of additive. Therefore, the FEEDAP Panel considers that Ronozyme® ProAct CT and L raise no concerns for the consumer of the products obtained from animals fed the additive.

3.2.4. Safety for user

The applicant submitted studies on the potential of the additive to cause eyes and skin irritation which were already assessed in a previous opinion (EFSA, 2009).

The skin/eye irritation potential of the additive was tested in studies GLP compliant performed according to OECD Guideline 404 and 405, respectively, which showed that the additive should be considered as irritant to human skin (category 2) and not an eye irritant.[34,35]

No study on skin sensitisation was provided. In the absence of data, no conclusions on the skin sensitisation potential can be reached.

The FEEDAP Panel concludes that the additive, in either form, is not an eye irritant but should be considered a skin irritant. Owing to the proteinaceous nature of the active substance it should be considered a respiratory sensitiser. In the absence of data, no conclusions on the skin sensitisation potential can be reached.

3.2.5. Safety for the environment

Viable cells of the production strain and DNA were not detected in a liquid concentrate representative of both final formulations.

The additive does not raise safety concerns for the environment with regard to the genetic modification of the production strain *B. licheniformis* DSM 19670.

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

[30] Technical dossier/Section III/ Annex 3-5.
[31] Technical dossier/Supplementary information January 2020/Submitted/B. safety/Appendix 9.
[32] Technical dossier/Section III/ Annex 3-3.
[33] Technical dossier/Section III/ Annex 3-4.
[34] Technical dossier/Section III/ Annex 3-6.
[35] Technical dossier/Section III/ Annex 3-7.

3.3. Efficacy

The applicant submitted three long-term trials in chickens for fattening,[36] one of which included a study of the apparent ileal digestibility of protein and energy. These studies have been already evaluated in a previous opinion (EFSA, 2009) where it was concluded that 'From the results of three growing trials, the potential efficacy of Ronozyme® ProAct CT/L has been demonstrated for chickens for fattening at the minimum recommended level of 15,000 PROT/kg. The data from the digestibility trial supports this conclusion'.

No new data has been provided which would lead the Panel to revise the previous conclusions. Therefore, the FEEDAP Panel concludes that Ronozyme® ProAct CT/L has the potential to be efficacious as a zootechnical additive in chickens for fattening at the recommended level of 15,000 PROT/kg feed.

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[37] and Good Manufacturing Practice.

4. Conclusions

The genetic modification of the production strain raises no concerns. Viable cells of the production strain and its DNA were not detected in a liquid concentrate representative of both final formulations.

The additive is safe for chickens for fattening at the recommended level of 15,000 PROT/kg feed.

The additive is safe for the consumers of food derived from animals fed with the additive.

The additive is not an eye irritant but should be considered a skin irritant and a respiratory sensitiser. In the absence of data, no conclusions on the skin sensitisation potential can be reached.

The use of the product as a feed additive is of no concern for the environment.

The additive has the potential to be efficacious in chickens for fattening at 15,000 PROT/kg feed.

5. Documentation as provided to EFSA/Chronology

Date	Event
19/02/2019	Dossier received by EFSA. RONOZYME® ProAct (serine protease EC 3.4.21.- produced by *Bacillus licheniformis* DSM 19670) for chickens for fattening. Submitted by DSM Nutritional Products Sp. z o.o. on behalf of DSM Nutritional Products Ltd.
11/03/2019	Reception mandate from the European Commission
11/06/2019	Application validated by EFSA – Start of the scientific assessment
02/07/2019	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, user safety*
23/08/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: methods of analysis*
11/09/2019	Comments received from Member States
04/11/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
28/01/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
22/04/2020	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*
19/06/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
22/07/2020	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*
15/10/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
27/01/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

[36] Technical dossier/Section IV/ Appendix 4-1. 4-2 and 4-3.

[37] 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

AMR	antimicrobial resistance
BIOHAZ	EFSA Panel on Biological Hazards
CFU	colony forming unit
EURL	European Union Reference Laboratory
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
GMO	EFSA Panel on Genetically Modified Organisms

LOD	limit of detection
LOQ	limit of quantification
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
QPS	qualified presumption of safety
WGS	whole genome sequence

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

RONOZYME® ProAct is the trade name of a *feed additive* containing as active substance *serine protease* (EC 3.4.21. - not yet fully classified) produced by *Bacillus licheniformis* (DSM 19670). This *feed additive* is currently authorized under the category/functional 4(a) "zootechnical additives"/ "digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/ 2003 (*feed additive* identification number 4a13). In the current application authorisation is sought under Article 4(1) for the use of the *feed additive* for chickens for fattening.

The *serine protease* activity is expressed in PROT units, where "one PROT is the amount of *serine protease* that liberates one micromol per minute of para-nitroaniline (pNA) from 1 mM Suc-Ala-Ala-Pro-Phe-pNA substrate at pH 9.0 and 37 °C". The *feed additive* is intended to be marketed in a solid (*RONOZYME® ProAct (CT)*) and a liquid (*RONOZYME® ProAct (L)*) form with the same minimum *serine protease* activity (75 000 PROT/g). *RONOZYME® ProAct* is intended to be incorporated directly or through *premixtures* at a minimum activity of *serine protease* of 15 000 PROT/kg *feedingstuffs*.

For the quantification of the activity of *serine protease* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted a single-laboratory validated and further verified colourimetric method, based on the enzymatic reaction of *serine protease* on the Suc-Ala-AlaPro-Phe-pNA substrate.

Based on the performance characteristics available the EURL recommends for official control this method for the quantification of the *serine protease* activity in the three target matrices. 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.

Annex B –

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(EFSA FEEDAP Panel, 2018a).[43]

(EFSA, 2007; EFSA BIOHAZ Panel, 2020).

[38] Technical dossier/Supplementary information October 2020/ProAct SIn answer EFSA August 2020 - Annex.pdf, replies to questions 1 and 2.
[39] Clarifications received by email on 06 November 2020.
[40] Technical dossier/Supplementary information October 2020/Annex 1.
[41] Technical dossier/Supplementary information October 2020/Annex 2.
[42] Technical dossier/Supplementary information October 2020/Annex 3.
[43] Technical dossier/ Supplementary information October 2020/Annex 4.