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SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of endo-β-1,4-xylanase produced by *Komagataella phaffii* CGMCC 7.371 (VTR-xylanase) for all avian species, piglets (suckling and weaned) and minor growing porcine species (Victory Enzymes GmbH)

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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 endo-β-1,4-xylanase (VTR-xylanase) as a zootechnical feed additive for all avian species, piglets (suckling and weaned) and minor growing porcine species. VTR-xylanase is available in a powder and a liquid form and is produced by a genetically modified strain of *Komagataella phaffii* (CGMCC 7.371). The genetic modification of the production strain does not give rise to safety concerns. Viable cells of the production strain and its DNA were not detected in the final products. The additive does not pose any safety concern regarding the production strain. VTR xylanase (powder/liquid) produced by *Komagataella phaffii* CGMCC 7.371 is safe for all avian species, piglets and minor growing porcine species at the proposed conditions of use. The use of both forms of the additive under assessment in animal nutrition under the proposed conditions of use raises no safety concerns for consumers or for the environment. The liquid and powder formulations of VTR-xylanase are non-irritant to eyes but should be considered skin sensitisers. No conclusions can be drawn on the potential of the final formulations of the additive to be irritant to skin. Due to the proteinaceous nature of the active substance, the additive is a respiratory sensitiser. The additive has the potential to be efficacious in all laying birds and piglets (suckling and weaned) from all *Suidae* at 2,000 U/kg and in all other avian species/categories at 1,000 U/kg feed.

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Keywords: zootechnical additives, digestibility enhancers, VTR-xylanase, endo-β-1,4-xylanase, *Komagataella phaffii* CGMCC 7.371, safety, efficacy

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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 feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Victory Enzymes GmbH[2] for the authorisation of the additive consisting of endo-β-1,4-xylanase produced by *Komagataella phaffii* CGMCC 7.371 (VTR-xylanase), when used as a feed additive for all avian species, piglets (weaned and suckling) and minor growing porcine species (category: zootechnical additive; functional group: digestibility enhancers).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 12 August 2021.

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 feed additive consisting of endo-β-1,4-xylanase produced by *Komagataella phaffii* CGMCC 7.371 (VTR-xylanase), when used under the proposed conditions of use (see **Section 3.1.6**).

1.2. Additional information

The additive has not been previously authorised for use in feed in the European Union.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of two technical dossiers[3] in support of the authorisation request for the use of endo-β-1,4-xylanase produced by *Komagataella phaffii* CGMCC 7.371 (VTR-xylanase liquid/powder) as a feed additive.

The dossier FAD-2021-0068 was received on 21 April 2021 and FAD-2021-0080 on 19 April 2021, and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00442 and https://open.efsa.europa.eu/questions/EFSA-Q-2021-00427, respectively.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' (elicitation) knowledge, 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 endo-1,4-β-xylanase in animal feed.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of endo-β-1,4-xylanase produced by *K. phaffii* CGMCC 7.371 (VTR-xylanase) is in line with the principles laid down in Regulation (EC) No 429/2008[5] 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 assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Victory Enzymes GmbH, Förschlag 3, D-91564, Neuendetteslau (Germany).
[3] FEED dossier references: FAD-2021-0068 and FAD-2021-0080.
[4] Evaluation report received on 02/06/2023 and available on the EU Science Hub: https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en.
[5] 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.

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on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (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 environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The product under assessment contains endo-β-1,4-xylanase (xylanase; Enzyme Commission (EC) 3.2.1.8) produced by *K. phaffii* CGMCC 7.371 and is intended to be used as a zootechnical additive (functional group: digestibility enhancers) in feed for all avian species, piglets (suckling and weaned) and minor growing porcine species. It will be hereafter referred to as VTR-xylanase (liquid and powder).

3.1. Characterisation

3.1.1. Characterisation of the production strain

The active substance (endo-β-1,4-xylanase) is produced by fermentation with a genetically modified strain of *Komagataella phaffi*, deposited in the China General Microbiological Culture Collection Center (CGMCC) with deposit number CGMCC 7.371.[6]

The taxonomical identification of the production strain CGMCC 7.371 as *K. phaffii* was confirmed by bioinformatic analysis of the whole genome sequence (WGS) data.[7] [redacted]

3.1.1.1. Information related to the genetically modified microorganism

Characterisation of the parental or recipient microorganism

The recipient strain is [redacted]

Description of the genetic modification

[redacted] The genetic modifications were analysed by aligning the genome of the production strain (*K. phaffii* CGMCC 7.371) against that of the recipient strain [redacted][7] [redacted]

The sequences introduced in the production strain do not raise safety concerns.

3.1.2. Manufacturing process

The enzyme is produced by fermentation with *K. phaffii* CGMCC 7.371.[8] [redacted]

[6] FAD-2021-0068/0080: Technical dossier/Section II/Annex_II_2_1_2_2.
[7] FAD-2021-0068/0080: Technical dossier/Section II/Annex_II_2_1_2_1.
[8] FAD-2021-0068/0080: Technical dossier/Section II/Annex II_3_1 and SIn_230622/Annex II_3_1.

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[redacted]

The applicant stated that no antimicrobials are used in the manufacturing process.

3.1.3. Characterisation of the additive

The additive is available in two formulations: VTR-xylanase powder, with a minimum xylanase activity of 100,000 U[9]/g; and VTR-xylanase liquid, with a minimum xylanase activity of 30,000 U/g.

Analytical data to confirm the specifications were provided for five batches of each formulation of the additive,[10] showing the following average values: 132,280 U (ranging 126,307–145,406)/g and 36,161 U (ranging 34,284–37,896)/g for the powder and liquid formulations, respectively. The content of TOS in three batches of the additive showed an average content of [redacted] for the powder and liquid formulations, respectively.[11]

Three batches of each formulation of the additive were analysed for chemical impurities and microbial contamination[12,13] The powder formulation showed an average value of 0.025 mg arsenic/kg (ranging 0.021–0.029) and of 0.021 mg lead/kg (ranging 0.017–0.025), whereas the contents of mercury and cadmium were below the respective limits of detection (LOD).[14] The liquid formulation showed an average value of 0.0021 mg lead/kg (ranging 0.017–0.025), whereas the contents of arsenic, mercury and cadmium were below the LOD.[15] [redacted][15]

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (dl-PCBs) were analysed in three batches of each formulation of the additive and found below the corresponding LOD, except for the content of dl-PCBs in two batches of the powder form, showing an average value of 0.0137 pg WHO-TEQ/g. The calculated (upper bound) levels of dioxins and the sum of dioxins and dl-PCBs for the powder form were 0.0852 and 0.0991 pg WHO-TEQ/g. For the liquid form, the calculated (upper bound) levels of dioxins and the sum of dioxins and dioxin-like-PCBs were 0.0852 pg WHO-PCDD/F-TEQ/g and 0.0986 pg WHO-PCDD/F-PCB-TEQ/g, respectively (in all three batches). The level of non-dioxin like PCBs was below LOD (0.3 ng/g). The analysis of mycotoxins, including aflatoxins (B1, B2, G1, G2),[16] fumonisins (B1, B2, B3), ochratoxin A, zearalenone, deoxynivalenol, and HT-2 toxin and T2 toxin, showed values below the LOQ[17] *Salmonella* spp. was not detected in 25 g of each of the three batches analysed for each form, Enterobacteriaceae, yeasts and moulds were below 10, 100 and 100 colony forming units (CFU)/g, respectively.

The FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

The presence of viable cells of the production strain in the final product was investigated in three batches of VTR-xylanase liquid and three batches of VTR-xylanase powder, each tested in triplicate.[18]

[redacted]

[9] One unit of xylanase activity (in terms of U) is defined as the amount of enzyme required to release one micromole of reducing sugar equivalents from arabinoxylan per minute at 40°C and pH 6.5.
[10] FAD-2021-0068/0080: Technical dossier/Section II/Annex II_1_3_3.
[11] FAD-2021-0068/0080: Technical dossier/Section II/Annex II_1_3_2.
[12] FAD-2021-0068/0080: Technical dossier/Section II/Annex II_1_4_3.
[13] FAD-2021-0068/0080: Technical dossier/Section II/Annex II_1_4_2.
[14] LOD (mg/kg) = As (0.002); Pb (0.0003); Hg (0.0007); Cd (0.0001).
[15] FAD-2021-0068/0080: Technical dossier/SIn_230622/Annex_2; LOQ = 10 mg/kg (powder)/1 mg/L (liquid).
[16] FAD-2021-0068/0080: Technical dossier/SIn_230622/Annex_2.
[17] LOQ (μg/kg) = aflatoxins (0.1), fumonisins (10); ochratoxin A (0.1); zearalenone (2); deoxynivalenol (10); HT-2 toxin (2) and T-2 toxin (2).
[18] FAD-2021-0068/0080: Technical dossier/Section II/Annex_II_2_2_2_2_1 and Annex_II_2_2_2_2_2.

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[redacted] Therefore, no viable cells of the production strain were found in VTR-xylanase liquid nor in VTR-xylanase powder.

The presence of recombinant DNA from the production strain in the final product was tested [redacted] in three batches of VTR-xylanase powder and three batches of VTR-xylanase liquid, each tested in triplicate.[19] [redacted] No recombinant DNA of the production strain was detected in VTR-xylanase powder nor in VTR-xylanase liquid.

3.1.4. Physical properties of the additive

The VTR-xylanase powder is a white to light grey powder solid preparation with an average solid density of 1,454 kg/m^3. The dusting potential of three batches of the powder form of the additive was determined using the Stauber-Heubach method and showed values on average of 21.7 mg/m^3 (ranging 0–35).[20] The particle size of the additive was analysed by laser-diffraction method; the results showed that, on average, 35%, 9.4% and 0.06% (v/v) of particles were < 100, < 50, < 10 μm, respectively.

The VTR-xylanase liquid is a yellowish to light brown liquid preparation with average viscosity of 2.01 cP at 20°C; and average bulk density of 1,100 kg/m^3.[21]

3.1.5. Stability and homogeneity

The shelf-life of the additive is claimed to be 18 months for powder and liquid formulations kept at 25°C or 5°C, respectively. The shelf-life of VTR-xylanase powder (3 batches) was studied when stored at 25 ± 1°C in silver non-transparent bags for up to 18 months. The enzyme activity at the end of the storage period was on average 79.0% (ranging 75.6–80.0) of the initial one.[22] The shelf-life of the VTR-xylanase liquid (3 batches) was studied when stored at 5 ± 1°C in brown non-transparent plastic bottles for up to 18 months. The enzyme activity at the end of the storage period was on average 95.9 (89.3–101.3) % of the initial one.[23] For the assessment of the stability of the additive in a premixture and complete feeds, one batch of VTR-xylanase powder was supplemented in a vitamin–mineral premixture for chickens for fattening at 2 g/kg premixture. The loss of xylanase activity of VTR-xylanase powder in the premixture tested after 1 and 6 months of storage at 25 ± 1°C in zip-lock plastic foil bags was of 41.2% and 61.2%, respectively.[24] The supplemented premixture was blended into two mash feeds for chickens for fattening and one for piglets to obtain a minimum xylanase activity of 2,000 U/kg feed. No losses were observed in any of the mash feeds after 3 months of storage in zip-lock plastic foil bags at 25°C.[25] A portion of the non-supplemented mash feeds (two for chickens and one for piglets) were pelleted (maximum temperature of 70°C) and the liquid form of the additive was sprayed onto them at 2,000 U/kg feed. Another portion was mixed with the premix supplemented with the powder form at 1% (w/w) to achieve a target activity of 2,000 U/kg feed and pelleted under similar conditions. The loss of activity observed after 3 months of storage in zip-lock plastic foil bags at 25°C ranged between 14.7% and 42.9% in the VTR-xylanase powder supplemented feeds and 9.7–23.3% for VTR-xylanase liquid sprayed feeds.

The homogeneous distribution of VTR-xylanase powder in mash and pelleted feed for chickens for fattening was studied in 10 subsamples. The coefficient of variation was 36.7% in mash feed and 30.3% in pellet, which is considered to be very high. For VTR-xylanase liquid sprayed on top of chickens for fattening pelleted feed the analysis of 10 subsamples showed a coefficient of variation of 6%.[25]

[19] FAD-2021-0068/0080: Technical dossier/SIn_230622/Annex_II_2_2_2_3_2_amd and Annex_II_2_2_2_3_1_amd.
[20] FAD-2021-0068/0080: Technical dossier/Section II/Annex II_1_5_1.
[21] FAD-2021-0068/0080: Technical dossier/Section II/Annex II_1_5_2.
[22] FAD-2021-0068/0080: Technical dossier/Section II/Annex II_4_1_1_1.
[23] FAD-2021-0068/0080: Technical dossier/Section II/Annex II_4_1_1_2.
[24] FAD-2021-0068/0080: Technical dossier/Section II/Annex II_4_1_2.
[25] FAD-2021-0068/0080: Technical dossier/Section II/Annex II_4_2.

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3.1.6. Conditions of use

The additive is intended to be used at the minimum proposed level of 2,000 U/kg complete feed for laying birds, and of 1,000 U/kg complete feed for other avian species/categories. As regards piglets (sucking and weaned) and minor growing porcine species, the minimum proposed level is 2,000 U/kg complete feed.

3.2. Safety

3.2.1. Safety of the production organism

The production organism belongs to *K. phaffii*, which is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment when used for enzyme production (EFSA BIOHAZ Panel, 2007, 2023). The production strain was identified as *K. phaffii* and differed from the parental strain [redacted] No complete genes of concern were introduced by the genetic modification. No viable cells or DNA of the production strain were detected in the final products. VTR-xylanase powder/liquid does not pose any safety concern regarding the production strain.

3.2.2. Toxicological studies

Toxicological studies are not required for fermentation products produced by a genetically modified microorganism for which the recipient strain is considered by EFSA to qualify for the QPS approach to safety assessment and for which the genetic modification raises no concerns. However, the applicant submitted the below toxicological studies to support the safety of the additive. All the toxicological studies were performed with the intermediate liquid concentrate form of xylanase ([redacted]) from which the two final VTR-xylanase formulations are obtained.

3.2.2.1. Bacterial reverse mutation test

In order to investigate the potential of the test item to induce gene mutations in bacteria, an Ames test was performed according to OECD Test Guideline (TG) 471 and claimed to follow good laboratory practice (GLP) [redacted][26] Therefore, the test item did not induce gene mutations in bacteria under the experimental conditions used in this study.

3.2.2.2. *In vitro* mammalian cell (human lymphocytes) micronucleus test

An *in vitro* micronucleus test was carried out to evaluate the potential of the test item to induce chromosome damage in human peripheral blood lymphocytes.[27] The study was performed in accordance with OECD TG 487 and claimed to follow GLP. [redacted] Therefore, the test item did not induce micronuclei in cultured human peripheral blood lymphocytes under the experimental conditions used in this study.

[26] FAD-2021-0068/0080: Technical dossier/Section III/Annex 2_2_2_1.
[27] FAD-2021-0068/0080: Technical dossier/Section III/Annex 2_2_2_2.

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3.2.2.3. Subchronic oral toxicity study

[redacted][28] The study was conducted in compliance with OECD TG 408 and claimed to follow GLP. [redacted] Therefore, under the conditions of the study, the no observed adverse effect level NOAEL for additive could be determined as [redacted] mg/kg body weight per day, the highest dose tested (equivalent to 80,392 U/kg bw per day).

3.2.2.4. Conclusions on the toxicological studies

The FEEDAP Panel concludes that the intermediate liquid concentrate used for the formulation of the additive showed no genotoxicity potential in tests addressing gene mutation and numerical and structural chromosome aberrations. Moreover, the results obtained in a subchronic oral toxicity study raised no concerns regarding the product and allowed to derive a NOAEL of 80,392 U/kg bw per day, the highest level tested.

3.2.3. Safety for the target species

No tolerance studies in relevant target species were submitted. In order to support the safety of the additive for the target species, the applicant referred to the subchronic oral toxicity study described above (see **Section 3.2.2**). The NOAEL identified (80,392 U/kg bw and day) was used to calculate the maximum safe level in chickens and turkeys for fattening, laying hens and piglets in accordance with the procedure described in the Guidance on the safety for the target species (EFSA FEEDAP Panel, 2017c); the results are shown in Table 1. The maximum safe levels obtained are higher than the recommended use level of 2,000 U/kg for piglets, minor growing porcine species and for laying birds and of 1,000 U/kg for other avian species/categories. Therefore, the Panel concludes that the additive is safe for all avian species, piglets (suckling and weaned) and minor growing porcine species at the proposed conditions of use.

Table 1: Maximum safe concentration of VTR-xylanase in the feed of the major target species

Animal category	Default values for daily feed intake (g DM per kg bw)	Maximum safe level in feed (U/kg complete feed)
Chickens for fattening	79	8,955
Turkeys for fattening	59	12,058
Laying hens	53	13,348
Piglets	44	16,078

3.2.4. Safety for the consumer

The enzyme is produced by a genetically modified strain of *K. phaffii*. This species is considered to qualify for the QPS approach to safety assessment when used for enzyme production provided the genetic modification raises no safety concerns. The identity of the strain was established, and the genetic modification of the production strain raises no concerns. Therefore, the production strain is presumed safe for production purposes and no concerns would raise for the consumer derived from the use of the additive in animal nutrition. The results obtained in the genotoxicity studies and the subchronic oral toxicity support this conclusion.

3.2.5. Safety for the user

3.2.5.1. Effect on respiratory system

The highest dusting potential measured in the solid formulation was 35 mg/m^3. Therefore, the exposure of the respiratory system to the additive is unlikely. Due to the proteinaceous nature of the active substance, the additive is considered a respiratory sensitiser.

[28] FAD-2021-0068/0080: Technical dossier/Section III/Annex 2_2_3.

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3.2.5.2. Effect on eyes and skin

The potential of the intermediate liquid concentrate used to formulate the final products to be irritant to the skin was investigated by performing an *in vitro* skin irritation study following the OECD TG 439.[29] Based on the results, the liquid intermediate enzyme concentrate is classified as non-irritant in accordance with the UN GHS 'No Category'.

The eye irritation potential of VTR-xylanase powder[30] and VTR-xylanase liquid[31] was investigated *in vitro* following the OECD TG 492. The results of the studies indicated that under the specified experimental conditions, both additives should be classified in accordance with the UN GHS as 'No Category'.

The skin sensitisation potential of VTR-xylanase powder[32] and VTR-xylanase liquid[33] was investigated *in vitro* following the OECD TG 442D. The results of these studies under the specified experimental conditions indicated that both formulations are skin sensitisers.

The skin sensitisation potential of VTR-xylanase powder[34] and VTR-xylanase liquid[35] was further investigated *in vitro* following the OECD TG 442E. The results of these studies showed that under the specified experimental conditions, both formulations are skin sensitisers.

3.2.5.3. Conclusions on safety for the user

Based on the results of the studies submitted, the FEEDAP Panel considered VTR-xylanase solid and VTR-xylanase liquid to be non-irritant eyes but should be considered skin sensitisers. No conclusions can be drawn on the potential of the final formulations of the additive to be irritant to skin. Due to the proteinaceous nature of the active substance, the additive is a respiratory sensitiser.

3.2.6. Safety for the environment

The production strain and its DNA were not detected in the final formulations of the additive. The active substance of the additive is a protein, and as such will be degraded/inactivated during the passage through the digestive tract of animals or in the environment. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

3.3. Efficacy

3.3.1. Efficacy for chickens for fattening

The applicant submitted three long-term trials aiming at assessing the effect of the additive on the zootechnical performance of chickens for fattening. All trials included a short-term balance study to assess the effect of the additive on the feed energy utilisation.

In trial 1,[36] 281-day-old male chickens for fattening (Ross 308) were distributed in 104 cages and randomly allocated to four dietary treatments (26 replicates per treatment, two birds per replicate). At day 21, half of the animals were removed from the trial and the remaining birds were raised in the same cages individually until the end of the trial. Two basal diets (starter from day 1 to 21; finisher from day 22 to 35) based on wheat, soya bean meal and rye were either not supplemented (control) or supplemented with VTR-xylanase (powder) to provide 1,000, 1,500 or 2,000 U/kg feed. The enzyme activity in the diets was confirmed analytically.[37] The experimental diets were offered ad libitum in pelleted form for 35 days. Mortality and health status were monitored daily and the most likely cause of death/culling recorded. Individual bird body weight and pen feed intake were monitored throughout the trial, and the average daily feed intake, average daily gain and feed to gain ratio were calculated and corrected for mortality. From day 14 to 19, total excreta were collected from each cage. The diet and excreta samples were analysed for dry matter and energy contents to calculate the apparent metabolisable energy (AME). The experimental data were subjected to ANOVA, including the diet as

[29] FAD-2021-0068/0080: Technical dossier/Section III/Annex 3_1_2.
[30] FAD-2021-0068/0080: Technical dossier/SIn_230622/Annex 3_1_2_2.
[31] FAD-2021-0068/0080: Technical dossier/SIn_230622/Annex 3_1_2_5.
[32] FAD-2021-0068/0080: Technical dossier/SIn_230622/Annex 3_1_2_3.
[33] FAD-2021-0068/0080: Technical dossier/SIn_230622/Annex 3_1_2_6.
[34] FAD-2021-0068/0080: Technical dossier/SIn_230622/Annex 3_1_2_4.
[35] FAD-2021-0068/0080: Technical dossier/SIn_230622/Annex 3_1_2_7.
[36] FAD-2021-0068: Technical dossier/Section IV/Annex 3_1.
[37] Enzyme activity (U/kg) for the starter/finisher diets: < 500/< 500 (Control); 1,400/2,200 (1,000); 2,100/2,450 (1,500); and 3,950/3,350 (2,000).

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fixed effect and the cage as the experimental unit. Group means were compared with Tukey-HSD test. Significance level was set at 0.05. Two birds were culled from the trial, one from the control and one from the 2,000 U/kg group. The zootechnical performance of the animals was not considered in the assessment because the animals were in cages, which does not reflect the standard farming practices in which the birds are raised in the EU. The AME was significantly higher in the groups of birds that received the additive at 1,000 U/kg feed and above in comparison with the control diet (12.5, 13.3, 13.1 and 13.2 MJ AME/kg feed, for the control, 1,000, 1,500 and 2,000 groups, respectively).

Trials 2[38] and 3[39] were performed following a similar design. In both trials, 540 one-day-old male chickens for fattening (Cobb 500) were distributed in 36 pens and randomly allocated to four dietary treatments (9 replicates per treatment, 15 birds per replicate). Two basal diets (starter – 1–14 days; and grower – 15–35 days) based on maize, soya bean meal and wheat were either not supplemented (control) or supplemented with the additive to provide 1,000, 1,500 or 2,000 U/kg feed. The enzyme activity of the diets was analytically confirmed.[40] The experimental diets were offered ad libitum in mash form for 35 days. Animal health and mortality were monitored daily and recorded throughout the trial. The body weight of the animals was measured at the start of the trial (day 1). Thereafter, body weight and feed intake were measured on weekly basis until day 35. The average daily feed intake, average daily gain and feed to gain ratio were calculated and corrected for mortality. At day 29, 18 birds per treatment were selected (based on body weights closest to the average of their corresponding treatment group) and moved to metabolic cages in pairs (9 replicates per treatment). From day 32 to 35, total excreta were collected and pooled per cage. The diet and excreta samples were analysed for dry matter, energy and nitrogen contents to calculate the nitrogen-corrected apparent metabolisable energy (AMEn). The experimental data were subjected to ANOVA, with the diet as fixed effect. The experimental unit used was the pen (performance parameters) or the cage (energy balance). Group means were compared with Tukey's test. Significance level was set at 0.05.

In trial 2, mortality was low and not treatment related. The animals receiving VTR-xylanase at 1,000 U/kg feed for 35 days showed higher final body weight and average daily gain, and better feed to gain ratio in comparison with the control diet. The dietary AMEn was higher in the groups receiving the additive at 1,500 and 2000 U/kg feed (Table 2).

Table 2: Effects of VTR-xylanase on the performance of chickens for fattening in Trial 2

Groups	Average daily feed intake	Final body weight	Average daily weight gain	Feed to gain ratio	AMEn	Mortality and culling
(U/kg feed)	(g)	(g)	(g)		MJ/kg	(%)
Control	86.0^{b}	1,928^{c}	53.8^{c}	1.60^{a}	11.43^{b}	2.2
1,000	86.9^{ab}	1,990^{b}	55.6^{b}	1.56^{b}	11.77^{ab}	2.2
1,500	87.6^{a}	2,017^{ab}	56.3^{ab}	1.56^{b}	11.90^{a}	1.5
2,000	88.1^{a}	2,033^{a}	56.8^{a}	1.55^{b}	11.91^{a}	2.2

(a,b,c): Mean values within a column with a different superscript are significantly different $p < 0.05$.

In trial 3, mortality including culling was 3, 1.5, 2.2 and 2.2% for the control, 1,000, 1,500 and 2,000, respectively. The zootechnical performance of the animals was lower than the expected for the breed under standard EU farming practices (68% of the expected); therefore, the results on the zootechnical performance were not considered further in the assessment. The AMEn was significantly higher in the group of birds receiving the additive from the intended level of 1,000 U/kg and above compared to control diet (10.5, 11.0, 11.5 and 11.7 MJ/kg for the control, 1,000, 1,500 and 2,000 groups, respectively).

Overall, the dietary supplementation of chickens for fattening with VTR-xylanase at 1,000 U/kg feed resulted in better energy utilisation of the diets in two of the trials and better zootechnical performance in a third one.

[38] FAD-2021-0068: Technical dossier/Section IV/Annex 3_2.
[39] FAD-2021-0068: Technical dossier/Section IV/Annex 3_3.
[40] Trial 2—U/kg for the starter/finisher diets: < 500/< 500 (Control); 1,400/1,500 (1,000); 640/1,900 (1,500); and 2,100/1,700 (2,000); Trial 3—U/kg for the starter/finisher diets: < 500/< 500 (Control); 1,200/2,100 (1,000); 2,315/1,300 (1,500); and 2,700/1,600 (2,000).

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3.3.2. Efficacy for laying hens

The applicant submitted three short-term trials aimed at studying the effect of the additive on the feed energy utilisation. The experimental design and the results on the energy utilisation are included in Table 3. In all cases, the birds were distributed in cages (trial 1) or pens (trials 2 and 3), which were randomly allocated to one of the four dietary groups, fed the basal diet either not supplemented (control) or supplemented with VTR-xylanase powder to provide 1,000, 1,500 or 2,000 U/kg feed.

Table 3: Trial design and use level of the efficacy trials performed in laying hens, and effect of the dietary supplementation with VTR-xylanase on the nitrogen-corrected apparent metabolisable energy

Trial	Total no. of animals (animals per replicate) Replicates per treatment	Breed Starting age (duration)	Composition feed (form)	Groups (U/kg feed)		AMEn
				Intended	Analysed	MJ/kg
1[41]	96 (3) 8	Tetra SL 23 weeks (28 days)	Wheat, soya bean meal and rye	0 1,000 1,500 2,000	< 500 760 1,600 2,500	11.71[b] 11.72[b] 11.95[ab] 12.01[a]
2[42]	200 (5) 10	Lohmann brown 22 weeks (63 days)	Wheat, soya bean meal and rye	0 1,000 1,500 2,000	< 500 1,400 2,700 2,850	10.85[b] 11.23[ab] 11.27[a] 11.32[a]
3[43]	200 (5) 10	Lohmann brown 24 weeks (63 days)	Wheat, soya bean meal and rye	0 1,000 1,500 2,000	< 500 1,300 1,300 3,000	10.32[b] 10.72[a] 10.91[a] 10.98[a]

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

Mortality and health status were monitored and the zootechnical performance parameters were recorded throughout the whole experimental period. In trial 1, from day 24 to 28, total excreta were collected daily and pooled by cage. The diet and excreta samples were analysed for dry matter, energy and nitrogen contents to calculate the AMEn. In trials 2 and 3, at day 57, 10 hens per treatment were moved to individual metabolic cages and allowed to adapt to the experimental conditions for 3 days. From day 60 to 63, total excreta were collected daily and pooled per cage. The diet and excreta samples were analysed for dry matter, energy and nitrogen contents to calculate the AMEn.

In the three trials, the experimental data were subjected to ANOVA, including the diet as fixed effect and the cage as the experimental unit. Mean groups were compared with Tukey-HSD test. Significance level was set at 0.05. No mortality was observed in any trial. No difference was seen on the zootechnical performance of the laying hens between groups. The supplementation of the feed with VTR-xylanase at 2,000, 1,500 and 1,000 U/kg resulted in higher dietary AMEn in trials 1, 2 and 3, respectively (Table 3); therefore, in all trials, the laying hens fed with VTR-xylanase at 2,000 U/kg feed showed higher energy utilisation of the diet in comparison with the control group.

3.3.3. Efficacy for weaned piglets

The applicant submitted three trials to support the efficacy of the additive in weaned piglets: one short-term balance trial aimed at assessing the effect of the additive on the energy utilisation of the diets (trial 1); and two long-term ones including balance trials, to assess both the effect on the zootechnical performance of the piglets and the energy utilisation of the diets (trials 2 and 3).

In trial 1,[44] 32 ca. 50-days-old castrated male weaned piglets (DanBred×Piétrain) were individually kept in metabolic crates and randomly allocated to four dietary treatments (8 replicates per treatment). The basal diet based on wheat, soya bean meal and rye was either not supplemented or

[41] FAD-2021-0068: Technical dossier/Section IV/Annex 2_1.
[42] FAD-2021-0068: Technical dossier/Section IV/Annex 2_2.
[43] FAD-2021-0068: Technical dossier/Section IV/Annex 2_3.
[44] FAD-2021-0080: Technical dossier/Section IV/Annex 2_1.

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supplemented with VTR-xylanase powder to provide 1,000, 1,500 or 2,000 U/kg complete feed. The enzyme activity of the diets was analytically confirmed.[45] The experimental diets were provided in pelleted form on a restricted basis (twice daily at 2.5 times the metabolisable energy (ME) requirement for maintenance) for 9 days. A balance was done with an adaptation period to the experimental conditions (crates and diets) of 4 days and a 5-day collection period in which total collection of faeces and urine was done and feed intake monitored. Mortality and health status were daily monitored, and the most likely cause of death or culling were recorded. The piglets were individually weighed on the first day under the experimental conditions (day 1) and at the end of the collection period (day 9), and the body weight gain was calculated. The feed, faeces and urine samples were analysed for dry matter and energy, and the ME content was calculated. The experimental data were subjected to ANOVA, including the diet as fixed effect. Group means were compared with Tukey's test. The significance level was set at 0.05. No mortality was observed during the experiment. No differences were observed on the performance parameters between groups. The supplementation of the diet of piglets at 2,000 U/kg feed resulted in higher ME content in comparison with the control (14.9, 15.0, 15.1 and 15.3 MJ ME/kg feed, for the control, 1,000, 1,500 and 2,000 groups, respectively).

In trials 2[46] and 3,[47] 80 25-day-old mixed weaned piglets (DanBred×Duroc) were distributed in 40 pens, with two piglets per pen (one male and one female) and randomly allocated to four dietary treatments (10 replicates per treatment). Two basal diets (starter, from day 1 to 14; and grower, from day 15 to 42) based on rye, soya bean meal and barley were either not supplemented (control) or supplemented with VTR-xylanase powder to provide 1,000, 1,500 or 2,000 U/kg complete feed. The enzyme activity of the diets was analytically confirmed.[48] The experimental diets were offered in mash form on ad libitum basis for 42 days. Mortality and health status were daily monitored and the most likely cause of death or culling were recorded. Average body weight per pen was measured at the beginning of the trial (day 1). Thereafter, body weight and feed intake of piglets were recorded weekly on a pen basis. The average daily feed intake, average daily gain and feed-to-gain ratio were calculated for each feeding period and the overall experiment. From day 39 to 42, faecal and urine samples were collected separately from each pen. The feed, faeces and urine samples were analysed for the content of dry matter and energy to calculate ME. Faeces were daily scored on a 0–3 scale (0 = normal; 3 = liquid faeces). The results were subjected to ANOVA, including the diet as fixed effect and using the pen as the experimental unit for the performance and digestibility studies. Group means were compared with Tukey's test. The significance level was set at 0.05. No mortality was observed in any trial.

In trial 2, the dietary supplementation of piglets with the additive showed better feed-to-gain ratio from 1,500 U/kg and higher daily gain at 2,000 U/kg in comparison with the control. In trial 3, it was observed a better feed-to-gain ratio and higher daily gain from 1,000, and higher final body weight from 1,500 U/kg compared to control (see Table 4). No differences were observed on the energy utilisation of the diet at any level in trial 2, while in trial 3, the inclusion of the additive from 1,500 U/kg showed higher dietary ME than the control diet. No differences were observed in the faecal scores between any group at any trial.

[45] Enzyme activity (U/kg): < 500 (Control); 1,100 (1,000); 1,600 (1,500); and 2,300 (2,000).
[46] FAD-2021-0080: Technical dossier/Section IV/Annex 3_1.
[47] FAD-2021-0080: Technical dossier/Section IV/Annex 3_2.
[48] Enzyme activity (U/kg) – Trial 2 (phase I/phase II): < 500/< 500; 1,300/1,200; 2,200/2,500; 1,900/3,300 for the control, 1,000, 1,500 and 2,000 U/kg diets, respectively; Trial 3 (phase I/phase II): < 500/< 500; 1,200/1,600; 1,900/1,800; 1,800/3,000 U/kg for the control, 1,000, 1,500 and 2,000 U/kg diets, respectively.

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Table 4: Effects of VTR-xylanase on the performance of weaned piglets and metabolisable energy content of feed

Trial	Groups	Daily feed intake	Initial body weight	Final body weight	Average daily weight gain	Feed to gain ratio	ME*
	(U/kg feed)	(g)	(kg)	(kg)	(g)		MJ/kg
2	0	824	7.15	27.4	481^{a}	1.71^{a}	12.7
	1,000	825	7.16	27.8	492^{ab}	1.68^{ab}	12.7
	1,500	827	7.15	28.2	500^{ab}	1.65^{b}	12.8
	2,000	830	7.15	28.3	505^{b}	1.64^{b}	12.9
3	0	757	8.07	27.7^{b}	467^{b}	1.62^{a}	13.1^{b}
	1,000	770	8.06	28.7^{ab}	491^{a}	1.57^{b}	13.3^{b}
	1,500	769	8.06	29.2^{a}	502^{a}	1.53^{bc}	13.6^{a}
	2,000	759	8.06	29.0^{a}	499^{a}	1.52^{c}	13.6^{a}

*: Metabolisable energy.
(a,b): Mean values within a trial and within a column with a different superscript are significantly different $p < 0.05$.

Overall, out of the three efficacy trials submitted in weaned piglets, one showed higher energy utilisation of the diet, a second one improved zootechnical performance and a third one both higher energy utilisation and better performance.

3.3.3.1. Conclusions on efficacy

According to the data provided, the Panel concludes that the additive has the potential to be efficacious as a zootechnical additive when added to feed in chickens for fattening at 1,000 U/kg and in laying hens and weaned piglets at 2,000 U/kg. The conclusions reached in chickens for fattening and laying hens can be extrapolated to all avian species at the corresponding recommended levels; and those in weaned piglets to suckling piglets (in the period in which solid feed is given) and minor porcine species at similar physiological development stages.

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[49] and Good Manufacturing Practice.

4. Conclusions

The production strain is a genetically modified strain of *Komagataella phaffii* (CGMCC 7.371). No viable cells nor DNA of the production strain were detected in the final product. The additive does not pose any safety concern regarding the production strain.

VTR-xylanase is safe for all avian species, piglets and minor growing porcine species at the proposed conditions of use.

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

VTR-xylanase is not irritant eyes but should be considered a skin and respiratory sensitiser. No conclusions can be drawn on the potential of the final formulation of the additive to be irritant to skin.

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

The FEEDAP Panel concludes that VTR-xylanase has the potential to be efficacious in laying birds and all *Suidae* (from suckling to weaning period) when added to feed at 2,000 U/kg feed, and in all other avian species/categories at 1,000 U/kg feed.

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[49] 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

AMEn	apparent metabolisable energy
ANOVA	Analysis of Variance
CGMCC	China General Microbiological Culture Collection Center
EURL	European Union Reference Laboratory
GLP	Good laboratory practice
LOD	limit of detection
LOQ	limit of quantification
ME	Metabolisable energy
OECD	Organisation for Economic Co-operation and Development
PCR	polymerase chain reaction
QPS	Qualified Presumption of Safety
WHO	World Health Organization

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