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Safety and efficacy of a feed additive consisting of endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase produced with *Talaromyces versatilis* IMI 378536 and DSM 26702 (ROVABIO[®] ADVANCE) for weaned piglets and pigs for fattening (ADISSEO France S.A.S)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of ROVABIO[®] ADVANCE (liquid and solid) which contains endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase. The enzymes present in the additive are produced by two strains of *Talaromyces versatilis*, one of them genetically modified. The additive is intended to be used as a feed additive for weaned piglets and pigs for fattening. Viable cells of the production strains and DNA of the genetically modified strain were not detected in the fermentation product used to formulate the additive. Based on the results of a tolerance trial in weaned piglets, the FEEDAP Panel concluded that ROVABIO[®] ADVANCE is safe for weaned piglets under the recommended conditions of use. This conclusion was extended to pigs for fattening. Based on the outcome of the toxicological studies performed, the Panel concluded that the additive is of no concern regarding consumer safety. ROVABIO[®] ADVANCE is not irritant to the skin or eyes but it is a skin and respiratory sensitiser. No risks to the environment are expected from the use of the additive in animal nutrition. Owing to the lack of sufficient data, the FEEDAP Panel could not conclude on the efficacy of ROVABIO[®] ADVANCE.

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Keywords: zootechnical additives, digestibility enhancers, xylanase, glucanase, *T. versatilis*, efficacy, safety

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1. Introduction

1.1. Background and Terms of Reference

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

The European Commission received a request from ADISSEO France S.A.S² for the authorisation of the additive consisting of endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase produced with *Talaromyces versatilis* IMI 378536 and DSM 26702 (ROVABIO® ADVANCE), when used as a feed additive for weaned piglets and pigs for fattening (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). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 24 March 2020.

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-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase produced with *Talaromyces versatilis* IMI 378536 and DSM 26702 (ROVABIO® ADVANCE), when used under the proposed conditions of use (see **Section 3.1.5**).

1.2. Additional information

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) issued three opinions on the safety and efficacy of an additive (ROVABIO® SPIKY) from the same applicant which has a similar composition and is produced by the same production strains. At this regard, the Panel assessed the safety and the efficacy of ROVABIO® SPIKY as a feed additive for chickens for fattening, chickens reared for laying and other minor poultry species (for fattening and reared for laying) (EFSA FEEDAP Panel, 2014). This opinion considered the safety aspects of the additive regarding the consumer, the user, the environment and the genetic modification of one of the production strains. Another opinion was adopted regarding the use of the additive as a feed additive for turkeys (EFSA FEEDAP Panel, 2015) and a last one regarding the use of the additive as a feed additive for all major and minor poultry species (EFSA FEEDAP Panel, 2016). The additive ROVABIO® SPIKY is currently authorised (4a22) for use in feed for chickens for fattening or reared for laying and minor poultry species for fattening/reared for laying,³ turkeys for fattening and for breeding⁴ and laying hens.⁵

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 ADISSEO FRANCE S.A.S. Immeuble Antony Parc 2, 10 Place du Général de Gaulle, 92160, Antony, France.

³ COMMISSION IMPLEMENTING REGULATION (EU) 2015/661 of 28 April 2015 concerning the authorisation of the preparation of endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase produced by *Talaromyces versatilis* sp. nov. IMI CC 378536 and *Talaromyces versatilis* sp. nov. DSM 26702 as a feed additive for chickens for fattening, chickens reared for laying and minor poultry species for fattening and reared for laying (holder of the authorisation Adisseo France S.A.S.). OJ L 110, 29.4.2015, p.1.

⁴ COMMISSION IMPLEMENTING REGULATION (EU) 2015/2304 of 10 December 2015 concerning the authorisation of a preparation of endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase produced by *Talaromyces versatilis* sp. nov. IMI CC 378536 and *Talaromyces versatilis* sp. nov. DSM 26702 as a feed additive for turkeys for fattening and for breeding (holder of the authorisation Adisseo France S.A.S.). OJ L 326, 11.12.2015, p.39.

⁵ COMMISSION IMPLEMENTING REGULATION (EU) 2017/210 of 7 February 2017 concerning the authorisation of a preparation of endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase produced by *Talaromyces versatilis* sp. nov. IMI CC 378536 and *Talaromyces versatilis* sp. nov. DSM 26702 as a feed additive for laying hens (holder of the authorisation Adisseo France S.A.S.). OJ L 33, 8.2.2017, p.19.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁶ in support of the authorisation request for the use of endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase produced with *Talaromyces versatilis* IMI 378536 and DSM 26702 (ROVABIO® ADVANCE) 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 or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' 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 the active substances in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁷

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of active substance (trade name of the product) is in line with the principles laid down in Regulation (EC) No 429/2008⁸ 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 on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP 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 additive, herein referred to as ROVABIO® ADVANCE, contains endo-1,4-beta-xylanase (xylanase, Enzyme Commission Number 3.2.1.8) and endo-1,3(4)-beta-glucanase (glucanase: Enzyme Commission Number 3.2.1.6) and is intended to be used as a zootechnical additive in feed for weaned piglets and pigs for fattening.

3.1. Characterisation

3.1.1. Characterisation of the production strains

The xylanase and glucanase present in the product are produced by two strains of *Talaromyces versatilis* (IMI 378536 and DSM 26702). [REDACTED]

[REDACTED] The two strains have been evaluated in previous opinions (EFSA FEEDAP Panel, 2013, 2014), and the main characteristics are presented here below.

The first strain is a non-genetically modified strain of *Talaromyces versatilis*⁹ which is deposited at the International Mycological Institute (now CABI) with the safe deposit number IMI 378536.¹⁰ [REDACTED]

[REDACTED]

⁶ FEED dossier reference: FAD-2020-0004.

⁷ The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2020-0004-rovabioadvance.pdf>

⁸ 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.

⁹ Formerly identified as *Penicillium funiculosum* <http://www.mycobank.org>

¹⁰ Technical dossier/Section II/Annex II.2.3 and Supplementary information December 2021/Q.1.a.

¹¹ <https://documents.com/d-the-uk-national-culture-collection-ukncc-biological-resource.pdf> and <http://www.indexfungorum.org/Publications/Index%20Fungorum%20no.26.pdf>

The second strain is a genetically modified strain of *Talaromyces versatilis* deposited at Deutsche Sammlung von Mikroorganismen und Zellkulturen with the deposit number DSM 26702.¹²

The applicant provided new data which taxonomically identified the two strains as *T. versatilis*

3.1.2. Manufacturing process

The enzymes contained in the additive are obtained from two products of fermentation each consisting of fed-batch fermentation, biomass filtration, concentration and purification. The resulting products are then mixed;

The liquid mixture (ROVABIO® ADVANCE SD) is either used directly to formulate the liquid form of the additive or spray dried to formulate the solid form. Final formulations include the addition of carriers and stabilisers. No antimicrobials of clinical relevance are used during the manufacture of the additive.

3.1.3. Characterisation of the additive

ROVABIO® ADVANCE is available in powder and liquid forms and contains two main enzyme activities, glucanase and xylanase. The analysis of the enzyme activities in the additive is performed following two methods, a viscosimetric-based method (viscosity units (U))¹⁴ or a method based on the colour formation of released sugars with 3,5-dinitrosalicylic acid (DNS U)¹⁵ (for further details, see Annex A).

ROVABIO® ADVANCE POWDER is a light- to dark-beige powder. The products of fermentation used represent 15–25% of the final additive and are mixed with 75–85 % wheat flour or maltodextrin (food grade). The minimum enzyme activities per gram of additive are 36,000 U or 3,740 DNS U of xylanase activity and 25,000 U or 2,600 DNS U of glucanase activity. The batch-to-batch variation was studied in five batches¹⁶ and showed mean values of xylanase of 56,994 U/g (coefficient of variation (CV) 2.3%) and 6,525 DNS U/g (CV 1.2%) and mean values of glucanase of 34,465 U/g (CV 1.9%) and 3,287 DNS U/g (CV 2.1%).

ROVABIO® ADVANCE LIQUID is a transparent to brown fluid that contains 4–8% (w/w) fermentation products, 10–30% sorbitol, 0.1–0.4% potassium sorbate and water up to 100%. The ingredients used to formulate the product are food grade.¹⁷ The product also contains sodium benzoate (< 0.1%). The minimum enzyme activities per gram of additive are 9,000 U or 940 DNS U of xylanase activity and 6,250 U or 650 DNS U of glucanase activity. The batch-to-batch variation was studied in five batches,¹⁶ which showed mean values for xylanase of 11,232 U/mL (CV 1.2%) and 1,425 DNS U/mL (CV 1.8%) and mean values for glucanase of 6,766 U/mL (CV 1.8%) and 730 DNS U/mL (CV 2.1%).

Three batches of each final formulation were analysed for chemical and microbial contamination.¹⁸ Lead, mercury, cadmium, fluorine and arsenic were not detected in all samples tested, with the

¹² Technical dossier/Section II/Annex II.2.10 and Supplementary information December 2021 Q.1.b.

¹³ Technical dossier/Supplementary information May 2021/Annex Q.1a.

¹⁴ One xylanase (or beta-glucanase) viscosity unit (U) is defined as the amount of xylanase (or beta-glucanase) that hydrolyses wheat arabinoxylan (or barley beta-glucan), reducing the solution viscosity, in order to change the relative fluidity by one dimensionless unit per minute, at pH 5.5 and 30°C.

¹⁵ One xylanase (or beta-glucanase) DNS unit corresponds to the amount of xylanase (or beta-glucanase) which liberates from the birchwood xylan (or barley beta-glucan) one µmol of xylose (or glucose) per minute at 50°C and pH 4.0 (or pH 5.0).

¹⁶ Technical dossier/Section II/Annex II.1.2.

¹⁷ Technical dossier/Section II/Annex II.1.1.

¹⁸ Technical dossier/Section II/Annex II.1.3 to II.1.7.

exception of cadmium in the solid formulation (0.02 mg/kg additive).¹⁹ The mean content of dioxins in ROVABIO® ADVANCE POWDER was 0.120 ng polychlorinated dibenzodioxins/-furans (PCDD/F) World Health Organization toxic equivalent (WHO-TEQ) (upper bound)/kg and the mean content of dioxins plus dioxin-like polychlorinated biphenyls (PCBs) was 0.185 ng PCDD/F+PCB WHO-TEQ (upper bound)/kg. For ROVABIO® ADVANCE LIQUID, the mean content of dioxins was 0.038 ng PCDD/F WHO-TEQ (upper bound)/kg and the mean content of dioxins plus dioxin-like PCBs is 0.06 ng PCDD/F+PCB WHO-TEQ (upper bound)/kg. All mycotoxins and other secondary metabolites tested were below the limit of detection and the measurements included aflatoxins B1, B2, G1 and G2, ochratoxin A, fumonisins B1, B2 and B3, zearalenone, diacetoxyscirpenol, neosalaniol, HT2-toxin, T2-toxin, nivalenol, deoxynivalenol, fusarenone-X, citrinine and patulin.²⁰ Microbial counts were all below the limit of detection (< 10 colony-forming units (CFU)/mL) for total aerobic counts, total *Enterobacteriaceae* coliforms, *Escherichia coli*, *Staphylococcus* coagulase positive, sulfate-reducing anaerobic microorganisms, *Clostridium perfringens*, yeasts and moulds, or absent in 25 g for *Salmonella* spp.²¹

Three batches of the liquid enzyme concentrate (ROVABIO® ADVANCE SD) used to formulate the additive were tested in triplicate for the presence of viable cells of the production strains.²²

No growth was observed. The positive controls (vegetative cells and spores) performed as expected.

Three batches of the liquid enzyme concentrate (ROVABIO® ADVANCE SD) used to formulate the additive were tested in triplicate for the presence of DNA from the genetically modified production strain.²³

No DNA was detected in the samples tested with a limit of detection between 0.1 and 10 ng/mL product.

The presence of antimicrobial activity was tested in 10% solutions of three batches of ROVABIO® ADVANCE SD liquid concentrate and in three batches of each of the two enzyme concentrates present in the concentrate.²⁴ A total of 10 strains of different bacteria²⁵ were used in the test. No antimicrobial activity was detected.

Three batches of ROVABIO® ADVANCE POWDER were studied for the dusting potential and particle size.²⁶ The mean dusting potential was 0.150 g/m³ (ranging 0.145–0.155).²⁷ Particle size distribution showed a mean of 18.4% of the particles with a diameter below 100 µm, 9.0% below 50 µm and 3% particles below 10 µm, with a mean particle size between 210 and 212 µm. The liquid formulation shows at 20°C a pH of 4.7, a relative density of 1.1 and a mean viscosity value at 30°C of 2.31 mPa·s.²⁸

3.1.4. Stability and homogeneity

The shelf-life of the two final formulations was studied in samples (30 g or mL) of three batches which were stored in closed low-density polyethylene zip bags (solid formulation) or in high-density polyethylene bottles (liquid formulation) at 5 or 20°C at 60% relative humidity for a total duration of 12 months.²⁹ The enzyme activities were measured as viscosity U and DNS U for the two enzymes. No losses on the enzyme activity were registered for the two formulations stored for 12 months at 5°C. After 12 months at 20°C, loss of initial enzyme activities (DNS and viscosity units) was ≤ 8% for the solid and ≤ 11% for the liquid.

¹⁹ Technical dossier/Section II/Annex II.1.3 and II.1.4. Limit of detection was for lead 0.02 in the solid or 0.05 mg/kg in the liquid, for mercury 0.005 mg/kg, for cadmium 0.01 mg/kg in the liquid, for fluorine 20 mg/kg and for arsenic 0.1 mg/kg.

²⁰ Technical dossier/Section II/Annex II.1.5. Limit of detection was 0.5 µg/kg for aflatoxins and ochratoxin A, 10 µg/kg for fumonisins and zearalenone, 20 µg/kg for patulin, and 50 µg/kg for all the other secondary metabolites.

²¹ Technical dossier/Section II/Annex II.1.6 and II.1.7.

²² Technical dossier/Supplementary information May 2021/Annex Q.7b and Q7bCoA.

²³ Technical dossier/Supplementary information May 2021/Annex Q.7a and Q7aCoA.

²⁴ Technical dossier/Supplementary information May 2021/Annex Q.6.

²⁵ *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, *Streptococcus pneumoniae* ATCC 49619, *Bacillus cereus* ATCC 11778, *Bacillus circulans* ATCC 4516, *Streptococcus pyrogenes* ATCC 12344, *Serratia marsencens* ATCC 14041.

²⁶ Technical dossier/Section II/Annex II.1.15.

²⁷ Technical dossier/Section II/Annex II.1.18.

²⁸ Technical dossier/Section II/Annex II.1.19.

²⁹ Technical dossier/Section II/Annex II.4.1.

The stability of ROVABIO® ADVANCE POWDER when added to two different complete premixture for pigs (with choline chloride) was studied in samples stored for 6 months at 20 or 30°C.³⁰ The results in the first premixture tested showed a mean loss of 24% for xylanase activity and 13% for glucanase when stored at 20°C, and 36% and 25% for samples stored at 30°C. The results in the second premixture showed no losses of enzyme activity in the samples stored at 20°C and losses of 15% and 10% for xylanase and glucanase activity, respectively, in the samples stored at 30°C.

The stability of ROVABIO® ADVANCE POWDER when added to mash feed for pigs (introduced via a premixture which contained choline chloride) was studied in samples stored for four months at two different temperatures (20 and 30°C) in closed packages.³¹ After 4 months at 20 or 30°C, loss in xylanase activity was 7 and 4%, respectively, and no losses were found for glucanase. The effect of heat treatment (at 70°C) was also investigated.³² Xylanase losses were 16% and no losses were found for the glucanase.

ROVABIO® ADVANCE LIQUID was sprayed onto pelleted feed for poultry for fattening at the recommended use level, and the stability during storage was investigated for four months at two different temperatures (20 and 30°C) in closed packages.³¹ After 4 months at 20 or 30°C, loss in xylanase activity was 7 and 4%, respectively, and for glucanase 8 and 2%, respectively.

The capacity to homogeneously distribute was established by analysing 10 samples of the feed used for the stability studies. The coefficient of variation was of 4.6% and 6.8% for the xylanase activity in the mash and pelleted diets and the corresponding coefficients for the glucanase were of 11.6% and 7.0%.

3.1.5. Conditions of use

The additive is intended for use in feed for weaned piglets and pigs for fattening at a minimum recommended level (in viscosity units) of 1,800 U xylanase and 1,250 U glucanase per kg feed. The solid formulation is to be incorporated into complete feed via a premixture and the liquid is to be sprayed onto the feed.

3.2. Safety

3.2.1. Safety of the genetic modification

The traits introduced by genetic modification in the production strain *T. versatilis* DSM 26702 [REDACTED], and are of no concern. Viable cells of the genetically modified production strain and its recombinant DNA were not detected in the fermentation product used to formulate the final additives. The additive does not give rise to safety concerns with regard to the genetically modified production strain DSM 26702.

3.2.2. Safety for the target species

The applicant provided a tolerance trial in weaned piglets to support the safety for the target species.³³

A total of 192 27-day-old weaned piglets (castrated males and females, Piétrain × (Large White × Landrace)) were distributed to 96 pens in groups of two animals (same sex). After 6 days of adaptation in which the animals received a commercial feed, the treatments were assigned to the pens. There were 3 dietary treatments with 32 replicates per treatment. Two basal diets were used, the starter was based on wheat, wheat bran, whey powder and soya bean meal and the grower diet was based on wheat, wheat bran, soybean meal and rapeseed meal. These diets were either not supplemented (control) or supplemented with ROVABIO® ADVANCE to provide 1,800 U xylanase and 1,250 U glucanase per kg feed (1× minimum recommended level) or 360,000 U xylanase and 250,000 U glucanase (200×) per kg feed (confirmed by analysis). Diets were offered in pelleted form and for 45 days (starter from 1 to 20 days and grower from 21 to 45).

Mortality and health status were checked every day and dead animals were necropsied. Animals were weighed on days 1, 21 and 45 (individually), feed intake was registered per pen and feed to gain ratio calculated. Statistical analyses were performed separately for males and females. Firstly,

³⁰ Technical dossier/Section II/Annex II.4.2 and Supplementary information May 2021/Annex Q8a to c.

³¹ Technical dossier/Section II/Annex II.4.3.

³² Technical dossier/Section II/Annex II.4.4.

³³ Technical dossier/Section III/Annexes III.1.1.A and B and Supplementary information May 2021 Annex III.1.1.

descriptive statistics of each variable were calculated, assessing normal distribution of data by using the Shapiro–Wilk test. After removal of outliers, the data were subject to non-inferiority testing with T-tests done by sex and considering the confidence margin in the control.

No animals died during the study. The initial body weight was 9 kg for all groups. The body weights at the end of the study were 37.3, 37.6 and 39.1 kg for control, 1× and 200× groups, respectively. The corresponding values for feed intake were 1.07, 1.03 and 1.10 kg feed/day/pig and for feed to gain ratio 1.70, 1.67 and 1.64, respectively. No adverse effects were observed in any of the performance parameters evaluated.

Based on the results in the tolerance trial in weaned piglets, the FEEDAP Panel concludes that ROVABIO® ADVANCE at the minimum recommended level is safe for piglets with a wide margin of safety. This conclusion is extended to pigs for fattening.

3.2.3. Safety for the consumer

The applicant submitted tests for each fermentation product [REDACTED] that were assessed in the context of a previous opinion (EFSA FEEDAP Panel, 2014) and included: an acute toxicity study (OECD TG 423),³⁴ a bacterial reverse mutation assay (OECD TG 471),³⁵ *in vitro* mammalian micronucleus tests (OECD TG 487),³⁶ an *in vivo* micronucleus test (OECD TG 474),³⁷ and subchronic oral toxicity study (OECD TG 408).³⁸ The tests did not identify a genotoxic potential in the fermentation products and no concerns were identified in the subchronic oral toxicity studies, and the FEEDAP Panel concluded based on the results obtained in the fermentation products that the additive is of no concern regarding consumer safety. The FEEDAP Panel reassessed the studies and considers that those conclusions apply to the current assessment.³⁹

3.2.4. Safety for the user

3.2.4.1. Effects on the respiratory system

No specific data were provided. The dusting potential of the solid formulation is 0.150 g/m³. Considering the proteinaceous nature of the active substances of the additive, it is considered a respiratory sensitiser.

3.2.4.2. Effects on skin and eyes

The applicant provided acute skin⁴⁰ and eye⁴¹ irritation studies done with ROVABIO® SPIKY in its solid and liquid formulations. The tests were assessed by the FEEDAP Panel in the context of another application and showed that the products are not irritant to skin or eyes (EFSA FEEDAP Panel, 2014). The formulations of ROVABIO® SPIKY and ROVABIO® ADVANCE differ slightly [REDACTED] resulting in a higher enzyme activity in ROVABIO® ADVANCE. However, the manufacturing of the two enzyme products is essentially the same, the strains are the same and the ingredient formulation of the final additives is similar. Therefore, the Panel considers that the conclusions from the tests done with ROVABIO® SPIKY apply to ROVABIO® ADVANCE.

A skin sensitisation test, local lymph node assay, was done in mice in accordance with OECD TG 429 with the mixture of the two enzyme fermentation products (ROVABIO® ADVANCE SD) that is used to formulate ROVABIO ADVANCE.⁴² Three groups of five female CBA/J mice were treated with 50 µL (25 µL per ear) of the test item at concentrations of 25%, 50% and 100%, while a fourth group of five females were treated with propylene glycol as a negative control. The stimulation index (based on the proliferation of lymphocytes in the draining auricular lymph nodes in treated groups vs. control) showed that the test item is a skin sensitiser. This conclusion is considered to apply to the final formulated products.

³⁴ Technical dossier/Section III/Annex 2.1.

³⁵ Technical dossier/Section III/Annex 2.2 and 2.5.

³⁶ Technical dossier/Section III/Annex 2.3, 2.6 and 2.7.

³⁷ Technical dossier/Section III/Annex 2.4 and 2.8

³⁸ Technical dossier/Section III/Annex 2.9 and 2.10

³⁹ Supplementary information May 2021 and December 2021.

⁴⁰ Technical dossier/Section III/Annexes III.3.1 and III.3.2.

⁴¹ Technical dossier/Section III/Annexes III.3.3 and III.3.4.

⁴² Technical dossier/Supplementary information December 2021/Annex Q8a

3.2.4.3. Conclusions on the safety for the user

ROVABIO® ADVANCE POWDER and LIQUID are not irritant to eyes or skin but are dermal and respiratory sensitisers.

3.2.5. Safety for the environment

No viable cells or DNA of the GMM production strain *Talaromyces versatilis* DSM 26702 were detected in the final products. The additive does not pose any environmental safety concern associated with the genetic modification of the production strain *Talaromyces versatilis* DSM 26702. The active substances present in the additive are proteins and as such will be degraded/inactivated during the passage through the digestive tract of animals. The other ingredients of the formulated products are considered to raise no concerns for the environment.

Considering all the above, no risk to the environment is expected, and no further environmental risk assessment is required.

3.3. Efficacy

3.3.1. Efficacy for weaned piglets

Three long-term trials were submitted. One of the trials was not considered further⁴³ due to the large number of animals that had to be treated (about 30%), mainly for respiratory and enteric disorders, which would reflect poor health status of the animals under study.

The other two studies followed a similar trial design, and the main elements are provided in Table 1. In the two trials, male and female weaned piglets were raised in pens separated by sex and two basal diets (pre-starter, starter) were either not supplemented (control) or supplemented with ROVABIO® ADVANCE to provide 1,800 xylanase viscosity units and 1,250 glucanase viscosity units per kg feed. The enzyme activities were confirmed by analysis. The animals received the feed in pelleted form for 42 days. Mortality and health status were checked throughout the study period. Piglet's body weight was measured at start and on days 14 and 42. Feed intake was recorded throughout the study period and feed to gain ratio was calculated for the different study periods. The data were analysed by an analysis of variance for each study and the treatment was considered as main effect. For study 1, sex and batch of animals were also considered in the model, as well as the possible interactions; mortality was analysed with the chi-square test and logistic regression. For study 2, the batch/room as a block and sex were also considered, along with the initial body weight as a covariate. The results of the two studies are presented in Table 2.

Table 1: Trial design and dosages of the efficacy trials performed in weaned piglets

Trial	Total No of animals (animals × replicate) replicates × treatment sex	Breed Age at start (duration)	Composition feed (Form) additive	Groups (xylanase-glucanase/kg feed)	
				Intended	Analysed ¹
1 ^(a)	576 18 16 (♀,♂castrated)	male P76 and female Topigs TN70 Landrace × Large White 28 days (42 days)	Wheat, wheat middlings, soybean meal, rapeseed meal, and whey powder in the first phase (pelleted) LIQUID	0	Not given
				1,800–1,250	1,861/1,604- 1,192/1,151
2 ^(b)	400 10 20 (♀,♂)	(Large White × Landrace) × (Piétrain × Duroc) 28 days (42 days)	Wheat, wheat middlings, soybean meal, rapeseed, rye and whey powder starter (pelleted) POWDER	0	–
				1,800–1,250	2,280/2,475- 1,447/1,246

1: Values are for xylanase and glucanase for pre-starter and starter diets.

(a): Technical dossier/Section IV/Annex IV.1.2 and supplementary information May 2021 Annex IV.1.2.

(b): Technical dossier/Section IV/Annex IV.1.4/supplementary information May 2021 Annex IV.1.4 and supplementary information December 2021/Annex Q.9.

⁴³ Technical dossier/Section IV/Annex IV.1.3 and Supplementary information May 2021 Annex IV.1.3.

Table 2: Effects of ROVABIO® ADVANCE on the performance of weaned piglets

Trial	Groups (xylanase-glucanase/kg feed)	Daily feed intake (kg)	Initial body weight (kg)	Final body weight (kg)	Feed to gain ratio	Mortality and culling (%)
1	0	584	7.4	21.2 ^b	1.78 ^a	4.6
	1,800–1,250	613	7.4	22.1 ^a	1.73 ^b	3.6
2	0	547	6.6	20.2 ^b	1.69	4.0
	1,800–1,250	574	6.6	21.4 ^a	1.65	6.5

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

The piglets that received ROVABIO® ADVANCE at the recommended level showed a higher final body weight in the two trials, as well as a better feed to gain ratio in one trial (Trial 1).

3.3.2. Efficacy for pigs for fattening

One short-term trial and three long-term trials were submitted for the assessment. The short-term trial was not considered further because the effect of the additive on the apparent metabolisable energy was not measured but only estimated.⁴⁴ The three long-term trials were also disregarded for the assessment: one due to the high amount of copper in the diet (160 mg Cu/kg feed);⁴⁵ another one due to the high number of animals requiring medical treatment (about 22%) and the mortality registered (5.4%);⁴⁶ and the third due to the use of medicated feed (amoxicillin and zinc) during the study period (for 21 days out of a total of 82).⁴⁷

3.3.3. Conclusions on efficacy

Weaned piglets receiving ROVABIO® ADVANCE at the minimum recommended level showed improvements in the final body weight of the animals in two trials coupled with a better feed to gain ratio in one of them. However, the data are not sufficient for the Panel to conclude on the efficacy of the additive in weaned piglets.

The Panel cannot conclude on the efficacy of the additive in pigs for fattening due to the lack of data.

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 Regulation⁴⁸ and Good Manufacturing Practice.

4. Conclusions

The genetic modification of the production strain *T. versatilis* DSM 26702 raises no safety concerns. No viable cells and no recombinant DNA of *T. versatilis* DSM 26702 were detected in the intermediate product that is used to formulate the additive.

ROVABIO® ADVANCE is safe for weaned piglets and pigs for fattening at the level of 1,800 xylanase viscosity units and 1,250 beta-glucanase viscosity units per kg feed.

The use of ROVABIO® ADVANCE as a feed additive raises no concerns for the consumers or for the environment.

ROVABIO® ADVANCE in either form is not an irritant to skin or eyes, but it is a dermal and a respiratory sensitiser.

⁴⁴ Technical dossier/Section IV/Annex IV.1.1 and Supplementary information May 2021.

⁴⁵ Technical dossier/Section IV/Annex IV.1.6 and Supplementary information May 2021 Annex IV.1.6 and Supplementary information December 2021.

⁴⁶ Technical dossier/Supplementary information May 2021/Annex IV.1.7 and supplementary information December 2021 Annex Q.12.

⁴⁷ Technical dossier/Section IV/Annex IV.1.5 and Supplementary information May 2021 Annex IV.1.5 and December 2021 Annex Q.10

⁴⁸ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

Owing to the lack of data, the FEEDAP Panel cannot conclude on the efficacy of the additive ROVABIO® ADVANCE in weaned piglets and in pigs for fattening.

5. Documentation provided to EFSA/Chronology

Date	Event
15/01/2020	Dossier received by EFSA. ROVABIO® ADVANCE powder and liquid for pigs for fattening and weaned piglets. Submitted by Adisseo France SAS.
11/02/2020	Reception mandate from the European Commission
24/03/2020	Application validated by EFSA – Start of the scientific assessment
20/05/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: methods of analysis, characterisation, safety for the target species and consumer, and efficacy.</i>
24/06/2020	Comments received from Member States
17/05/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
06/11/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
06/08/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation, safety for the target species, consumer and user, and efficacy.</i>
06/12/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
23/03/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Aquilina G, Bories G, Chesson A, Cocconcelli PS, de Knecht J, Dierick NA, Gralak MA, Gropp J, Halle I, Hogstrand C, Kroker R, Leng L, Puente SL, Haldorsen AKL, Mantovani A, Martelli G, Mezes M, Renshaw D, Saarela M, Sejrsen K and Westendorf J, 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 3 pp. <https://doi.org/10.2903/j.efsa.2012.2539>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific opinion on Rovabio® Excel (endo-1,3(4)-beta-glucanase and endo-1,4-beta-xylanase) as a feed additive for chickens and turkeys for fattening, laying hens, piglets (weaned) and pigs for fattening, ducks, guinea fowls, quails, geese, pheasants and pigeons. EFSA Journal 2013;11(7):3321, 26 pp. <https://doi.org/10.2903/j.efsa.2013.3321>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014. Scientific Opinion on the safety and efficacy of Rovabio® Spiky (endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase) as a feed additive for chickens for fattening, chickens reared for laying and other minor poultry species (for fattening and reared for laying). EFSA Journal 2014;12(7):3793, 20 pp. <https://doi.org/10.2903/j.efsa.2014.3793>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific opinion on the safety and efficacy of ROVABIO® spiky (endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase) as a feed additive for turkeys and minor poultry species for fattening or reared for laying or breeding. EFSA Journal 2015;13(5):4106, 9 pp. <https://doi.org/10.2903/j.efsa.2015.4106>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. Scientific opinion on the safety and efficacy of ROVABIO® SPIKY (endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase) as a feed additive for all major and minor poultry species. EFSA Journal 2016;14(10):4510, 10 pp. <https://doi.org/10.2903/j.efsa.2016.4510>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Dujardin B, Galobart J and Innocenti ML, 2017a. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 45 pp. <https://doi.org/10.2903/j.efsa.2017.5022>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML, 2017b. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 45 pp. <https://doi.org/10.2903/j.efsa.2017.5023>

- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017c. Guidance on the assessment of the safety of feed additives for the target species. *EFSA Journal* 2017;15(10):5021, 55 pp. <https://doi.org/10.2903/j.efsa.2017.5021>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2018a. Guidance on the assessment of the efficacy of feed additives. *EFSA Journal* 2018;16(5):5274, 35 pp. <https://doi.org/10.2903/j.efsa.2018.5274>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018b. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal* 2018;16(3):5206, 55 pp. <https://doi.org/10.2903/j.efsa.2018.5206>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Bastos M, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brock T, de Knecht J, Kolar B, van Beelen P, Padovani L, Tarres-Call J, Vettori MV and Azimonti G, 2019. Guidance on the assessment of the safety of feed additives for the environment. *EFSA Journal* 2019;17(4):5648, 45 pp. <https://doi.org/10.2903/j.efsa.2019.5648>

Abbreviations

FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
LOQ	limit of quantification

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for ROVABIO® ADVANCE

In the current application, an authorisation is sought under Article 4(1) for Rovabio® Advance, under the category/functional 4(a) 'zootechnical additives'/ 'digestibility enhancers' according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the feed additive for pigs for fattening and weaned piglets.

According to the Applicant, the feed additive contains two active substances: endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase, produced from *Talaromyces versatilis* (IMI 378536) and (DSM 26702), respectively. The enzymatic activity for both enzymes is expressed by the Applicant in viscosity (U) and DNS units, where:

- One xylanase (or beta-glucanase) viscosity unit (U) is defined as the amount of xylanase (or beta-glucanase) that hydrolyses wheat arabinoxylan (or barley beta-glucan), reducing the solution viscosity, in order to change the relative fluidity by one dimensionless unit per minute, at pH 5.5 and 30°C
- One xylanase (or beta-glucanase) DNS unit corresponds to the amount of xylanase (or beta-glucanase) which liberate from the xylan (or barley beta-glucan) substrate one micromol of xylose (or glucose) per minute at 50°C and pH 4.0 (or pH 5.0)

The product is intended to be marketed as powder (Rovabio® Advance POWDER) and liquid (Rovabio® Advance LIQUID) formulations, having the following guaranteed minimum activities:

- for xylanase: 36,000 U/g or 3,740 DNS units/g and 9,000 U/mL or 940 DNS units/mL, and
- for beta-glucanase: 25,000 U/g or 2,600 DNS units/g and 6,250 U/mL or 650 DNS units/mL.

The feed additive is intended to be incorporated into premixtures and/or complete feedingstuffs to obtain a minimum xylanase and beta-glucanase activities of 1,800 and 1,250 viscosity U/kg, respectively.

When expressing the enzymatic activity in terms of viscosity units, a viscometric method is applied, while for the enzymatic activity in DNS units, a colorimetric method is used.

For the quantification of xylanase and beta-glucanase activities in the feed additive, premixtures and feedingstuffs, the Applicant submitted single-laboratory validated and further verified viscometric methods. In these methods, the endo-1,4-beta-xylanase (or the endo-1,3(4)-beta-glucanase) catalyses the hydrolysis of xylosidic (or glycosidic) bonds in the wheat arabinoxylan (or barley beta-glucan) substrate to yield xylose (or glucose) and reduces consequently the viscosity of the sample solution.

Furthermore, the Applicant submitted alternative colorimetric (DNS) methods for the quantification of xylanase and beta-glucanase activities in the feed additive. The colorimetric methods are based on the enzymatic hydrolysis of the xylan (or barley beta-glucan) substrate by xylanase (or beta-glucanase) and the consequent colour formation of the released sugars with 3,5-dinitrosalicylic acid (DNS) at pH 4.0 (or pH 5.0) and 50°C.

Based on the acceptable performance characteristics, the EURL recommends for official control (1) the single-laboratory validated and further verified viscometric methods for the determination of xylanase and beta-glucanase in the feed additive, premixtures and feedingstuffs and (2) the alternative colorimetric (DNS) methods for the determination of xylanase and beta-glucanase in the feed additive.

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.