Adopted: 30 January 2024

DOI: 10.2903/j.efsa.2024.8614

efsa JOURNAL

SCIENTIFIC OPINION

Safety and efficacy of a feed additive consisting of fumonisin esterase produced with *Komagataella phaffii* NCAIM (P) Y001485 for all pigs (piglets, pigs for fattening, sows and minor growing and reproductive porcine species) (Dr. Bata 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** | **Mojca Durjava** | **Maryline Kouba** | **Marta López-Alonso** | **Secundino López Puente** | **Francesca Marcon** | **Baltasar Mayo** | **Alena Pechová** | **Mariana Petkova** | **Fernando Ramos** | **Roberto Edoardo Villa** | **Ruud Woutersen** | **Giovanna Martelli** | **Rosella Brozzi** | **Jaume Galobart** | **Matteo Lorenzo Innocenti** | **Jordi Ortuño** | **Elisa Pettenati** | **Fabiola Pizzo** | **Jordi Tarrés-Call** | **Maria Vittoria Vettori** | **Anita Radovnikovic**

Correspondence: feedap@efsa.europa.eu

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of the additive based on fumonisin esterase (Free Yeast® F), produced with a genetically modified strain of *Komagataella phaffii*. The additive is categorised as a technological feed additive, for the reduction of the contamination of feed by mycotoxins and intended for use in all pigs species (piglets, pigs for fattening, sows and minor growing and reproductive porcine species). It was shown that the production strain and its recombinant genes are not present in the additive. The FEEDAP Panel concluded that the additive is safe for weaned and suckling piglets and pigs for fattening, and all minor growing porcine species up to 60 U/kg complete feed. No conclusions can be drawn on the safety of the additive in sows. The use of the additive in animal nutrition is of no concern for consumer safety. The additive is dust-free, and therefore, respiratory sensitisation/irritation is unlikely. The additive is non-irritant to the eyes and the skin. No conclusion could be made on skin sensitisation. The use of the additive as a feed additive is considered safe for the environment. The Panel concluded that the additive is efficacious as technological feed additive for the reduction of feed contamination by fumonisins, when used at the minimum recommended concentration of 60 U/kg. This conclusion can be extrapolated to all growing and reproductive pigs and other minor porcine species.

KEYWORDS

Free Yeast® F, fumonisin esterase, *Komagataella phaffii*, mycotoxins reduction, technological additive

CONTENTS

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 (Dr Bata Ltd)[2] for the authorisation of the additive consisting of fumonisin esterase (EC 3.1.1.87) produced with *Komagataella phaffii* NCAIM (P) Y001485, for use in all pigs (piglets, pigs for fattening, sows and minor growing and reproductive porcine species) (category technological additives; functional group: substances for reduction of the contamination of feed by mycotoxins).

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 dossier was received on 27.05.2021 and the general information and supporting documentation are available on OpenEFSA at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00470. The particulars and documents in support of the application were considered valid by EFSA as of 22 October 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 under assessment, when used under the proposed conditions of use (see **Section 3.1.8**).

1.2 | Additional information

The product under assessment is an enzyme-based additive, containing fumonisin esterase (EC 3.1.1.87) produced with genetically modified strain of *K. phaffii*, intended to decrease the level of fumonisin mycotoxins that may be found as contaminants in feed. The additive under assessment is not currently authorised in the EU.

The FEEDAP Panel delivered four opinions on the safety and efficacy of fumonisin esterase (EC 3.1.1.87) produced with different strains of *Komagataella phaffii* (formerly *K. pastoris*) (EFSA FEEDAP Panel, 2014, 2016, 2018a, 2020).

Fumonisin esterase (EC 3.1.1.87) produced with *Komagataella phaffii* (DSM 32159) is authorised as technological additive for all animal species (1m03i).[3] Fumonisin esterase (EC 3.1.1.87) produced with *Komagataella pastoris* (DSM 26643) is currently authorised as technological feed additive for use in pigs[4] and avian species (1m03).[5]

2 | DATA AND METHODOLOGIES

2.1 | Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[6] in support of the authorisation request for the use of fumonisin esterase (EC 3.1.1.87) produced with *Komagataella phaffii* NCAIM (P) Y001485 as feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 22 October 2021 to 22 January 2022. for which the received comments were considered for the assessment.

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 substance/agent in animal feed/marker residue in tissues.[7]

[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]Dr Bata Ltd, Bajcsy-Zs. Str 139, 2364 Ocsa, Hungary.

[3]Commission Implementing Regulation (EU) 2021/363 of 26 February 2021 concerning the authorisation of a preparation of fumonisin esterase produced by Komagataella phaffii DSM 32159 as a feed additive for all animal species.

[4]Commission Implementing Regulation (EU) No 1115/2014 of 21 October 2014 concerning the authorisation of a preparation of fumonisin esterase produced by Komagataella pastoris (DSM 26643) as a feed additive for pigs. OJ L 302, 22.10.2014, p. 51.

[5]Commission Implementing Regulation (EU) 2017/913 of 29 May 2017 concerning the authorisation of a preparation of fumonisin esterase produced by Komagataella pastoris (DSM 26643) as a feed additive for all avian species. OJ L 139,30.5.2017, p. 33.

[6]FEED dossier reference: FAD-2021-0055.

[7]The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2021-0055_en

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the additive is in line with the principles laid down in Regulation (EC) No 429/2008[8] 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 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, 2018b), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018c), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3 | ASSESSMENT

The additive containing fumonisin esterase (EC 3.1.1.87) produced with *K. phaffii* NCAIM (P) Y001485 is intended to be used as a technological additive (functional group: substances for reduction of the contamination of feed by mycotoxins) in feed for all pigs (piglets, pigs for fattening, sows and minor growing and reproductive porcine species). The proposed claim is to reduce the contamination of fumonisin B1 (FB1) and related fumonisins in feeds.

3.1 | Characterisation

3.1.1 | Characterisation of the active substance

The active substance is the enzyme fumonisin esterase (EC 3.1.1.87), which can degrade FB1 by cleavage of the ester bonds and the release of the tricarballylic acid (TCA) and hydrolysed (HFB1) (Figure 1). In case of incomplete hydrolysis, partially hydrolysed fumonisin can be formed (PHFB1). The activity of fumonisin esterase (EC 3.1.1.87) is expressed in fumonisin esterase units (U).[9]

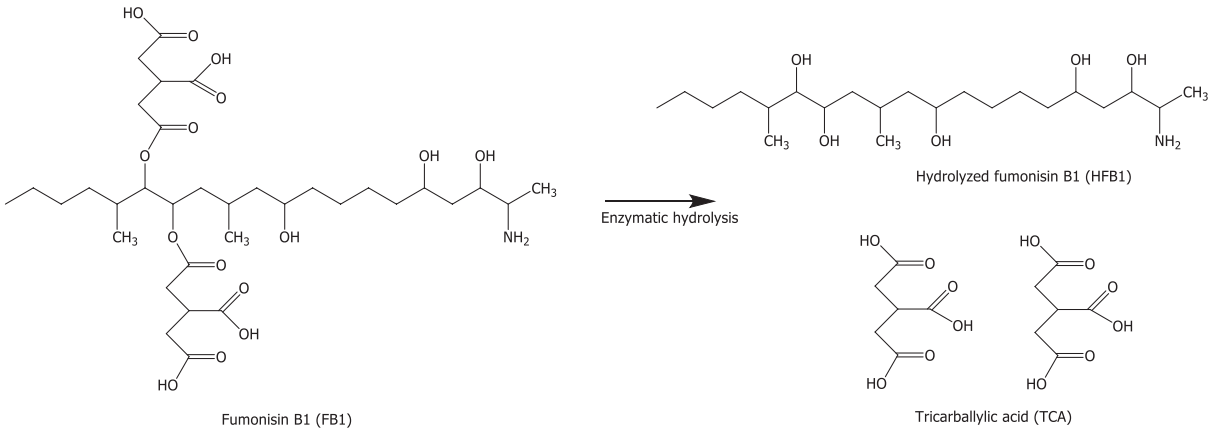

FIGURE 1 Degradation of FB1 by fumonisin esterase (EC 3.1.1.87).

3.1.2 | Characterisation of the production microorganism

Fumonisin esterase (EC 3.1.1.87) is produced with a genetically modified strain of *Komagataella phaffii* which has been deposited in the Hungarian National Collection of Agricultural and Industrial Microorganisms (NCAIM) with the accession number NCAIM (P) Y001485.[10]

The production strain was identified[11] as *K. phaffii* by whole genome sequencing (WGS) analysis which showed an average nucleotide identity of > 99% with the [redacted]

[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]One unit (U) is the amount of enzyme that releases one μmol of HFB1 per minute from 100 μM of FB1 in 50 mM phosphate buffer pH 6.0 at 37°C.

[10]Technical dossier/Section II/Annex 2.2.1.2/1.

[11]Technical dossier/Section II/Annex 2.2.1.2/3.

3.1.2.1 | *Information related to the genetically modified microorganism*

Characterisation of the recipient or parental microorganism

The production strain was derived [redacted][12].

Characteristics of the donor organisms

The fumonisin esterase (EC 3.1.1.87) protein sequence is derived from [redacted]

[redacted][13]

3.1.3 | Manufacturing process

Fumonisin esterase (EC 3.1.1.87) is produced with *K. phaffii* NCAIM (P) Y001485. [redacted][14]

The carrier used is a feed material, a commercially available inactivated dried brewer's yeast[15] and contains non-viable *Saccharomyces cerevisiae*.[16]

3.1.4 | Characterisation of the additive

The additive is composed of [redacted] fumonisin esterase (EC 3.1.1.87) and of inactivated *Saccharomyces cerevisiae* (up to 100%) as a carrier. The additive is specified to contain a minimum activity of fumonisin esterase (EC 3.1.1.87) of 1200 U/g.[17]

Analytical data to confirm the specification were provided for five batches of the additive, showing an average value of fumonisin esterase (EC 3.1.1.87) activity of 1306 U/g (range: 1262–1377 U/g).[18] Humidity content was in average 10.4% (range 10.3%–10.6%).

Three batches of the additive were analysed for impurities.[19] Cadmium was 0.06 mg/kg; lead, mercury and arsenic were below the limit of quantification (LOQ) of the analytical methods.[20] Aflatoxins (B1, B2, G1, G2), deoxynivalenol, zearalenone,

[12]Technical dossier/Section II /Annex 2.2.1.2.
[13]Technical dossier/Supplementary information 2023-01-12/Annex SIn_3.
[14]Technical dossier/Supplementary information 2023-01-12/Annex SIn_6.
[15]Technical dossier/ Section II/ ANNEX 2.3.2.
[16]COMMISSION REGULATION (EU) 2017/1017 of 15 June 2017 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials.
[17]Technical dossier/Section II Characterisation.
[18]Technical dossier/Section II/Annex 2.1.3.
[19]Technical dossier/Section II/Annex 2.1.4. and Supplementary information 2023-01-12/Annex Sin 1 and APPENDIX SIn1/1.
[20]Tested according to EPA method. LOQ(As) = 0.09 mg/kg; LOQ(Hg) = 0.02 mg/kg; LOQ(Cd) = 0.02 mg/kg; LOQ(Pb) = 0.09 mg/kg (12% moisture)

T2-toxin, HT2-toxin and fumonisins (B1, B2, B3) levels were below the LOQ of the analytical methods[21]; ochratoxin A content was 0.001 mg/kg (LOQ).

The applicant tested three batches of the additive for microbiological contamination.[22] Results showed that Enterobacteriaceae were < 10 CFU/g, *E. coli* < 10 CFU/g, yeasts and moulds < 10 CFU/g and *Bacillus cereus* < 10 CFU/g. *Salmonella* was not detected in 25 g in any of the batches tested.

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

The presence of viable cells of the production strain was tested in three independent batches of the fermentation product used to formulate the additive (7275–7388 U/g), each tested in triplicate.[23] Proper controls were included. Samples of 10 mL were diluted in 100 mL of sterile water and filtered using a 0.45-µm membrane. After filtration, the filter was placed on agar medium containing zeocin and incubated for a total of 48 h at 28°C. No colonies were detected in any of the samples.[24]

The presence of DNA of the production strain was tested in three samples of the fermentation product used to formulate the additive (7249–7312 U/g), each tested in triplicate.[25] Two sets of primers were used, one targeting 89 bp of the zeocin resistance gene and the other one targeting 82 bp of the *Arg4* gene. The analysis started with 100 mL of samples. A quantitative PCR was used. The DNA extraction method included a mechanical lysis procedure. No DNA was detected in the samples.[26] The detection limit was below 1 pg/mL.

3.1.5 | Physical properties of the additive

The additive appears as light brown homogeneous granulate. [redacted][27]

3.1.6 | Stability and homogeneity

The shelf-life of the additive was studied in three batches of the additive stored at 25°C/60% relative humidity (RH) or 40°C/75% RH for 24 months.[28] Negligible losses were observed on the enzyme activity at the end of storage period at both temperatures.

Stability of the additive in one batch of a vitamin–mineral premixture[29] (for pigs for fattening) containing the additive at 0.5% was tested when stored at 25°C/60% RH for 6 months. No loss in the fumonisin esterase activity (6475 U/kg) was observed at the end of the storage period.

The stability of the additive when incorporated in mash or pelleted pig feed was studied at two supplementation levels of fumonisin esterase (EC 3.1.1.87) (60 and 360 U/kg). The mash feed was pelleted[30] at 86°C, and both mash and pelleted feed were stored in paper bags at 25°C for 3 months. Pelleting of feed did not significantly reduce the fumonisin esterase (EC 3.1.1.87) activity in any of the feeds. Losses in enzyme activity after 3 months storage were 0% and 9.3% for the mash feed supplemented with 60 and 360 U/kg, respectively, while corresponding values for the pelleted feed were 4.6% and 11.1%.

The capacity for homogenous distribution of the additive was studied in 10 samples of a commercial feed for pigs (63 U/kg).[31] The coefficient of variation was 7.6%.

3.1.7 | Interference with the analysis of mycotoxins in feed

The possible interference of the additive in the determination of fumonisins in feedstuffs was examined in the feeds used for the efficacy trials.[32]

[21]Limit of quantification (LOQ) (total aflatoxins (B1, B2, G1, G2)) = 0.004 mg/kg, LOQ (deoxynivalenol) = 0.1mg/kg, LOQ (zearalenone) = 0.010 mg/kg, LOQ(T2) = 0.01 mg/kg, LOQ(HT2) = 0.010 mg/kg, LOQ (fumonisin B1) = 0.05 mg/kg LOQ (fumonisin B2) = 0.05 mg/kg; LOQ (fumonisin B3) = 0.05 mg/kg; LOQ (fumonisin (B1 + B2 + B3)) = 0.150 mg/kg, LOQ (ochratoxin A) = 0.001 mg/kg.

[22]Technical dossier/Supplementary information 2023-01-12/Annex Sin_2.

[23]Technical dossier/Supplementary information 2023-01-12/Annex Sin_4.

[24]Technical dossier/Section II/Annex 2.2.1.2/6.

[25]Technical dossier/Supplementary information 2023-01-12/Annex Sin_5.

[26]Technical dossier/Supplementary information 2023-04-11/Annex Sin2_1.

[27]Technical dossier/Section II/Annex 2.1.5.

[28]Technical dossier/Section II/Annex 2.4.1.1.

[29]Technical dossier/Section II/Annex 2.4.1.2 and Annex M.

[30]Technical dossier/Supplementary information 2023-01-12/Annex SIn 7.

[31]Technical dossier/Section II/Annex 2.4.2.

[32]Technical dossier/Section II/ Section 2.4.4.

The analytical determination of fumonisins in contaminated feeds (4.4–4.7 mg/kg feed) was not affected by the addition of additive in feed (60 U/kg).[33] Given the specificity of the fumonisin esterase (EC 3.1.1.87), it is highly unlikely that this enzyme would present an interference in the analytical determination of other classes of mycotoxins.

3.1.8 | Conditions of use

The additive is intended[34] to be used in feed for all pigs (piglets, pigs for fattening, sows and minor growing and reproductive porcine species) at an inclusion level from 60 to 360 U/kg complete feed.

3.2 | Safety

3.2.1 | Safety of the production microorganism

The production organism belongs to *K. phaffii*, a species that is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment when used for enzyme production (EFSA, 2007; EFSA BIOHAZ Panel, 2023). [redacted] However, since viable cells and DNA of the production strain were not detected in the fermentation product used to formulate the final product, it can be concluded that the additive does not pose any safety concern for the target species, consumer and the environment, with regard to the production strain.

3.2.2 | Safety for the target species

The applicant provided one tolerance study in weaned piglets[35] and one in sows[36] to support the safety of the additive for the target species. The tolerance study in sows was not further considered as abnormally high levels of mortality and culling (due to health reasons) were observed in the control group (22%).

3.2.2.1 | *Weaned piglets*

A total of 187 hybrid[37] mixed male and female weaned piglets (ca. 25 days old; average initial body weight = 6.2 kg) were distributed in 21 pens (with 8/9 piglets each) and randomly allocated to three dietary groups (7 replicates/group). Two basal diets (pre-starter, from day 1 to 17; starter, from day 18 to 42) based on barley, triticale, maize and soybean meal were either not supplemented (control) or supplemented with the additive to provide fumonisin esterase activity of 65 (1.1× minimum use level) or 6500 (110×) U/kg feed. The fumonisin esterase (EC 3.1.1.87) activity in the diets was analytically confirmed.[38] The experimental diets were offered ad libitum in mash form for 42 days.

The mortality and health status of the animals were monitored daily. Animals were weighed individually at the beginning and end of the experiment, and the feed intake recorded for each feeding phase (pre-starter/starter). The average daily gain and the feed-to-gain ratio were calculated for the whole experimental period. The effect of the inclusion of the additive in the diet was evaluating by means of two one-sided tests (TOST), assessing whether the difference between control and the use level (1.1×)/overdose (110×) groups fell within the calculated 90% confidence intervals.

No animal died during the trial. The supplementation of the feed of weaned piglets with the additive at 1.1× or 110× the minimum recommended level for 42 days showed to be equivalent for all the productive parameters measured to the control (control values for final body weight = 22.9 kg; average daily gain = 398 g; cumulative feed intake = 31.0 kg; feed-to-gain ratio = 1.88).

Based on the data of the tolerance trial, the Panel concludes that the additive is safe for weaned piglets at 60 U/kg feed. This conclusion is extended to suckling piglets and pigs for fattening, and extrapolated to minor growing porcine species.

The Panel notes that this conclusion is limited to the minimum use level of the additive providing fumonisin esterase activity at 60 U/kg. Based on the tolerance trial design, in order to conclude on the maximum use level (360 U/kg), data on blood haematology and biochemistry should have been provided in line with the Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c).

[33] [redacted]

[34] Technical dossier/Supplementary information 2023-12-05 and Supplementary information 2023-01-12.

[35] Technical dossier/Supplementary information 2023-01-12/Annex Sin 10.1.

[36] Technical dossier/Supplementary information 2023-01-12/Annex Sin 10.2.

[37] Topigs-Norsvin 070 × DanBred.

[38] Fumonisin esterase activity (pre-starter/starter; U/kg feed): control = <LOD; 1.1× minimum use level = 67/69 U/kg; 110× minimum use level = 6860/6897 U/kg feed.

3.2.2.2 | *Conclusions on safety for the target species*

The additive is safe up to 60 U/kg complete feed for weaned and suckling piglets and pigs for fattening, and all minor growing porcine species. In the absence of adequate data, the Panel cannot conclude on the safety of the additive at the maximum proposed use level (360 U/kg) nor on the safety for sows and minor reproductive porcine species.

3.2.3 | Safety for the consumer

The production organism belongs to a species, *K. phaffii*, that is considered by EFSA to be suitable for the QPS approach and the final product raised no safety concerns for the consumer with regard to the production strain (see Section 3.2.1). Therefore, no concerns are expected from residues of the fermentation process/production strain remaining in the final product.

The function of the enzyme fumonisin esterase (EC 3.1.1.87) is to breakdown the structure of fumonisins, resulting in metabolites of possible toxicological concern. The applicant carried out an extensive literature search (ELS) to investigate the toxicological profile of fumonisin metabolites.

3.2.3.1 | *Metabolites of fumonisins and their toxicity*

Fumonisin esterase (EC 3.1.1.87) can hydrolyse two ester bonds in FB1 and related fumonisins, releasing the two tricarballylic acid (TCA) moieties and an aliphatic aminopentol, referred to as HFB1. PHFB1 can also be released, when only one of the ester bonds is cleaved.

An ELS[39] was conducted by the applicant to identify any relevant information on the potential toxicity of the breakdown products of the enzymatic degradation of FB1 by the additive. [redacted]

The FEEDAP Panel noted that some of the publications provided by the applicant in the current dossier have been already reviewed either by the FEEDAP Panel (2014) (e.g. Gelderblom et al., 1993; Grenier et al., 2012; Humpf et al., 1998; Russell & Forsberg, 1986; Schwartz et al., 1988; Seiferlein et al., 2007) or by the EFSA CONTAM Panel (2018) (Caloni et al., 2002; Collins et al., 2006; Gelderblom et al., 1993; Grenier et al., 2012; Hahn et al., 2015; Howard et al., 2002; Voss et al., 2009).

In 2014, the FEEDAP Panel concluded that the metabolites of FB1 have a lower toxicity than the parent compound (EFSA FEEDAP Panel, 2014). The CONTAM Panel (EFSA CONTAM Panel, 2018) concluded, based on in vivo studies, that HFB1 and PHFB1 have a similar toxicological profile to FB1, but have lower toxicological potency. However, the actual potency could not be accurately quantified.

The review of the studies from the ELS showed that no new studies concerning the toxicological profile of TCA and PHFB1, other than those previously evaluated by the FEEDAP Panel (EFSA FEEDAP Panel, 2014) and CONTAM Panel (EFSA CONTAM Panel, 2018) were available. Two relevant publications on HFB1, which have not been mentioned in previous EFSA opinions but which support the conclusions drawn there, are presented below.

[redacted]

Considering all the above, the FEEDAP Panel concludes that the metabolites produced by the action of fumonisin esterase (EC 3.1.1.87) on FB1 show lower toxic potency compared to the parent compound and that no toxicological concern for the consumers is resulting.

3.2.3.2 | *Conclusions on safety for the consumer*

The FEEDAP Panel concludes that the use of the additive in feed for pigs is safe for the consumers.

3.2.4 | Safety for the user

3.2.4.1 | *Effects on the respiratory system*

No specific information on the effect of the additive to the respiratory system was submitted by the applicant. Due to the proteinaceous nature of the additive, it is considered a respiratory sensitiser. The additive is dust-free, and therefore, exposure of users by inhalation is unlikely.

[39]Technical dossier/Section III/ Annex 3.2.3.1. and Supplementary information 2023-11-3.

3.2.4.2 | *Effect on eyes and skin*

The skin irritation potential of the additive was tested in a study performed according to OECD Testing Guideline (TG) 431, which showed that it is not a skin irritant.[40]

The eye irritation and corrosivity potential of the additive were tested in a study performed according to OECD TG 438. The results of this test were inconclusive.[41]

As a further investigation, an eye irritation study was performed in vivo according to the OECD TG 405.[42] The additive caused a slight conjunctival redness which was fully reversible within 48 h. Based on these results, the test item is considered to be non-irritant for the eye under the conditions of the test.

The skin sensitisation potential of the additive was tested in a study performed according to OECD TG 429, which showed that it is not a dermal sensitiser.[43] The FEEDAP Panel notes that the OECD test guidelines available at present are designed to assess the skin sensitisation potential of chemical substances only and that currently no validated assays for assessing the sensitisation potential of inactivated microorganisms are available. Therefore, no conclusions can be drawn on the skin sensitisation potential of the additive.

3.2.4.3 | *Conclusions on safety for the user*

Due to the proteinaceous nature of the additive, it is considered to be a respiratory sensitiser. However, the additive is dust free, and therefore, the exposure through inhalation is unlikely. The additive is not a skin nor eye irritant. No conclusions could be drawn on its potential to be a dermal sensitiser.

3.2.5 | Safety for the environment

The active substance, fumonisin esterase (EC 3.1.1.87), is a protein and as such will be largely degraded/inactivated in the digestive tract of animals. In addition, any hydrolysis of fumonisins in the digestive tract would simply anticipate naturally occurring degradation of fumonisins in soils. The production strain and its DNA were not detected in the additive; therefore, its use will not give rise to any environmental safety concerns associated with the genetic modification of the production strain. The FEEDAP Panel considers that no risks for the environment are expected following the use of the additive in feedingstuffs under proposed conditions of use.

3.3 | Efficacy

The applicant submitted one in vitro and nine in vivo trials: five in weaned piglets[44] and four in sows[45] to support the efficacy of the additive.

3.3.1 | In vitro study

The applicant provided a study[46] to support the mechanism of action of the fumonisin esterase (EC 3.1.1.87) by means of the de-esterification of FB1 into a HFB1 and two TCA moieties. The additive was mixed at different concentrations with a solution containing FB1 (100 μM FB1). The reaction was stopped at different timepoints (30, 60 and 90 min) by boiling for 5 min and the reaction mixture was analysed[47] for the content of FB1 and HFB1. The results showed a time-dependent reduction of the starting compound (FB1) and increased formation of HFB1, which after 90 min resulted in almost complete hydrolysis of the starting fumonisin.

Although the hydrolysing efficacy of the additive has been shown against FB1, the enzyme fumonisin esterase (EC 3.1.1.87) is assumed to be equally effective in its action against all recognised fumonisins (EFSA FEEDAP Panel, 2016, 2018a).

3.3.2 | Efficacy for weaned piglets

The applicant submitted five short-term trials in weaned piglets. A summary of the experimental design is presented in Table 1 and the main results in Table 2.

[40]Technical dossier/Supplementary information 2023-01-12/Annex SIn 11.
[41]Technical dossier/Section III/Annex 3.3 Appendix 3.3/1.
[42]Technical dossier/Section III/Annex 3.3 Appendix 3.3/2.
[43]Technical dossier/Section III/Annex 3.3 Appendix 3.3/3.
[44]Technical dossier/Section IV/Annex IV_4.2.1.1., Annex IV_4.2.1.2. and Annex IV_4.2.1.3. and Supplementary information_2023-09-12/Annex SIn_3.1. and Annex SIn_3.3.
[45]Technical dossier/Section IV/Annex IV_4.2.2.1., Annex IV_4.2.2.2. and Annex IV_4.2.2.3.; Supplementary information_12-09-2023/Annex SIn_3.2.
[46]Technical dossier/Section IV.
[47][redacted].

TABLE 1 Trial design of the efficacy trials performed in weaned piglets and levels of fumonisin and fumonisin esterase (EC 3.1.1.87) in feed.

Trial	Total n° of animals (animals × replicate) replicates × treatment	Breed Body weight Gender	Composition feed (form)	Groups	Fumonisin (intended) mg/kg feed	Fumonisin[b] (analytically confirmed) mg/kg	Fumonisin esterase (intended) U/kg feed	Fumonisin esterase (analytically confirmed) U/kg feed
1	[redacted]	[redacted]	[redacted]	UU	–	< 1.0	–	–
	[redacted]	[redacted]	[redacted]	CU	ca. 4.5	4.37	–	–
	[redacted]	[redacted]	[redacted]	CS	ca. 4.5	4.37	60	67
2	[redacted]	[redacted][a]	[redacted]	UU	–	< 1.0	–	–
	[redacted]	[redacted]	[redacted]	CU	ca. 4.5	4.49	–	–
	[redacted]	[redacted]	[redacted]	CS	ca. 4.5	4.63	60	66
3	[redacted]	[redacted]	[redacted]	UU	–	< 1.0	–	–
	[redacted]	[redacted]	[redacted]	CU	ca. 4.5	4.25	–	–
	[redacted]	[redacted]	[redacted]	CS	ca. 4.5	4.69	60	73
4	[redacted]	[redacted][a]	[redacted]	UU	–	< 1.0	–	–
	[redacted]	[redacted]	[redacted]	CU	ca. 4.5	4.73	–	–
	[redacted]	[redacted]	[redacted]	CS	ca. 4.5	4.82	60	67
5	[redacted]	[redacted]	[redacted]	UU	–	< 1.0	–	–
	[redacted]	[redacted]	[redacted]	CU	ca. 4.5	4.58	–	–
	[redacted]	[redacted]	[redacted]	CS	ca. 4.5	4.57	60	66

Abbreviations: CU, contaminated and unsupplemented; CS, contaminated and supplemented; UU, uncontaminated and unsupplemented.

[a]Topigs-Norsvin070 × Danbred.

[b]Average concentration of fumonisin measured in pre-starter and starter diet.

In all trials, the piglets were distributed in pens, which were randomly allocated to one of the three experimental groups based on the contamination with fumonisins and the supplementation with the additive: a group receiving the basal diet uncontaminated and unsupplemented (UU); a group receiving the basal diet contaminated with fumonisin at approximately 4.5 mg/kg feed (range 4.25–4.73 mg/kg) and unsupplemented (CU); and a third group receiving the basal diet contaminated with fumonisin at approximately 4.6 mg/kg feed (range 4.37–4.82 mg/kg) and supplemented with the additive[48] at 60 U/kg complete feed (CS). [redacted][49] The level of fumonisins and the enzyme activity[50] in the feed were analytically confirmed (see Table 2). In all trials, the experimental diets were offered to the animals ad libitum for 28 days.

Health and zootechnical parameters were monitored throughout the experiment. At the start (day 1) and end (day 28) of the trials, blood was sampled from all piglets and analysed for the content of sphinganine (Sa) and sphingosine (So). The sphinganine/sphingosine ratio (Sa/So) was calculated (Sa/So is the most sensitive end point for fumonisin toxicosis). In trial 1, faecal samples were collected from individual animals[51] and analysed[52] for the content of FB1, HFB1 and PHFB1. [redacted]

The Panel notes that in trials 2 and 3, due to the low sample size ($N = 3$), the statistical analysis used was not adequate and the use of non-parametric testing would have been preferred for the statistical analyses, as indicated in the Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b).

[48]Technical dossier/Supplementary information 2023-01-12/Annex Sin 15.

[49][redacted]

[50]Technical dossier/Supplementary information 2023-01-12/Annex Sin 14 and Supplementary information 2023-09-12/Sln 3.1-Sin 3.3.

[51]Technical dossier/Supplementary information 2023-01-12/Annex Sin 13.

[52]Technical dossier/Section IV/Annex 4.2.4; [redacted].

TABLE 2 The effect of fumonisin esterase (EC 3.1.1.87) on the serum sphinganine/sphingosine ratio (Sa/So) and the concentration of fumonisin B1 (FB1) and its breakdown product (HFB1) in faeces of weaned piglets.

Trial	Groups	Fumonisin (intended) (mg/kg feed)	Fumonisin esterase (intended) (U/kg feed)	Sa/So Day 1 (serum) –	Sa/So Day 28 (serum) –	FB1 Day 28 (faeces) (μg/g)	HFB1 Day 28 (faeces) (μg/g)
1	UU	–	–	0.147	[redacted]*	[redacted]*	[redacted]*
	CU	ca. 4.5	–	0.147	[redacted]	[redacted]	[redacted]
	CS	ca. 4.5	60	0.151	[redacted]*	[redacted]*	[redacted]*
2	UU	–	–	0.151	[redacted]*	n/a	n/a
	CU	ca. 4.5	–	0.150	[redacted]		
	CS	ca. 4.5	60	0.160	[redacted]*		
3	UU	–	–	0.162	[redacted]*	n/a	n/a
	CU	ca. 4.5	–	0.174	[redacted]		
	CS	ca. 4.5	60	0.158	[redacted]*		
4	UU	–	–	0.145	[redacted]*	n/a	n/a
	CU	ca. 4.5	–	0.144	[redacted]		
	CS	ca. 4.5	60	0.142	[redacted]*		
5	UU	–	–	0.137	[redacted]*	n/a	n/a
	CU	ca. 4.5	–	0.139	[redacted]		
	CS	ca. 4.5	60	0.141	[redacted]*		

Abbreviations: CS, contaminated and supplemented; CU, contaminated and unsupplemented; n/a, not analysed; UU, uncontaminated and unsupplemented.
*Values in a column in a given trial are significantly different CU vs. CS and CU vs. UU (Dunnett's test).

In trial 1, one piglet died and another one was culled, both belonging to the same pen from the UU group. In trial 4, one piglet from UU and two from CU died. No mortality or culling were observed during any of the other trials. The productive performance was not affected either by the fumonisin contamination or the enzyme supplementation in any trial.

In all trials, the Sa/So ratio on day 28 was increased in the CU group compared to the UU group. The supplementation of the feeds with the additive at the minimum use level (60 U/kg feed) showed significantly lower Sa/So ratio in serum compared to the CU group. In trial 1, faecal concentration of FB1 was significantly reduced in the CS group in comparison with the CU and the concentration of HFB1 was significantly increased in the CS group when compared to the CU group.

3.3.3 | Efficacy for sows

The applicant submitted four short-term trials in sows to support the efficacy of the additive. The studies followed a similar design as the one described above for piglets. A summary of the experimental design is presented in Table 3 and the main results in Table 4.

TABLE 3 Trial design of the efficacy trials performed in sows and levels of fumonisin and fumonisin esterase (EC 3.1.1.87) in feed.

Trial	Total n° of animals (animals × replicate) Replicates × treatment	Breed Start body weight Parity	Composition feed (form)	Groups	Fumonisin (intended) mg/kg feed	Fumonisin[b] (analytically confirmed) mg/kg feed	Fumonisin esterase (intended) U/kg feed	Fumonisin esterase (analytically confirmed) U/kg feed
1	[redacted]	[redacted]	[redacted]	UU	–	< 1.0	–	–
	[redacted]	[redacted]	[redacted]	CU	ca. 4.5	4.40	–	–
	[redacted]	[redacted]	[redacted]	CS	ca. 4.5	4.40	60	71
2	[redacted]	[redacted][a]	[redacted]	UU	–	< 1.0	–	–
	[redacted]	[redacted]	[redacted]	CU	ca. 4.5	4.67	–	–
	[redacted]	[redacted]	[redacted]	CS	ca. 4.5	4.61	60	69
3	[redacted]	[redacted]	[redacted]	UU	–	< 1.0	–	–
	[redacted]	[redacted]	[redacted]					
	[redacted]	[redacted]	[redacted]	CS	ca. 4.5	4.14	60	65
4	[redacted]	[redacted][a]	[redacted]	UU	–	< 1.0	–	–
	[redacted]	[redacted]	[redacted]	CU	ca. 4.5	4.81	–	–
	[redacted]	[redacted]	[redacted]	CS	ca. 4.5	4.72	60	70

Abbreviations: CU, contaminated and unsupplemented; CS, contaminated and supplemented; UU, uncontaminated and unsupplemented.
[a]Topigs-Norsvin070 × Danbred.
[b]Average concentration of fumonisin in pre-starter and starter diet.

The sows were housed individually (trials 1, 2 and 4) or in pairs (trial 3) and randomly allocated to one of the three experimental groups based on the contamination with fumonisins and the supplementation with the additive: a group receiving the basal diet uncontaminated and unsupplemented (UU); a group receiving the basal diet contaminated with fumonisin at approximately 4.6 mg/kg feed (range 4.40–4.81 mg/kg) and unsupplemented (CU); and a third group receiving the basal diet contaminated with fumonisin at approximately 4.4 mg/kg feed (range 4.14–4.72 mg/kg) and supplemented with the additive at 60 U/kg complete feed (CS). [redacted] The level of fumonisins and the enzyme activity in the feed were analytically confirmed (see Table 4). In all trials, the experimental diets were offered to the animals ad libitum for 28 days.

Health and zootechnical parameters were monitored throughout the trial. At the start (day 1) and end (day 28) of the trials, blood was sampled from all sows and analysed for the content of Sa and So, and the sphinganine/sphingosine ratio (Sa/So) was calculated. In trial 1, faecal samples were collected from the cleaned pen floors into faeces collection tubes[53] on days 1 and 28 and analysed for the content of FB1 and HFB1. [redacted]

The Panel notes that in trial 3, due to the low sample size ($N = 3$), the statistical analysis used was not adequate, and the use of non-parametric testing would have been preferred, as indicated in the Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b).

TABLE 4 The effect of fumonisin esterase (EC 3.1.1.87) on the serum sphinganine/sphingosine ratio (Sa/So) and the concentration of FB1 and its breakdown product (HFB1) in faeces of sows.

Trial	Groups	Fumonisin (intended) (mg/kg feed)	Fumonisin esterase (intended) (U/kg feed)	Sa/So Day 1 (serum)	Sa/So Day 28 (serum)	FB1 Day 28 (faeces) (μg/g)	HFB1 Day 28 (faeces) (μg/g)
1	UU	–	–	0.163	[redacted]*	[redacted]*	[redacted]*
	CU	ca. 4.5	–	0.168	[redacted]	[redacted]	[redacted]
	CS	ca. 4.5	60	0.166	[redacted]*	[redacted]*	[redacted]*
2	UU	–	–	0.145	[redacted]*	n/a	n/a
	CU	ca. 4.5	–	0.137	[redacted]		
	CS	ca. 4.5	60	0.146	[redacted]*		
3	UU	–	–	0.160	[redacted]*	n/a	n/a
	CU	ca. 4.5	–	0.173	[redacted]		
	CS	ca. 4.5	60	0.172	[redacted]*		
4	UU	–	–	0.143	[redacted]*	n/a	n/a
	CU	ca. 4.5	–	0.155	[redacted]		
	CS	ca. 4.5	60	0.152	[redacted]*		

Abbreviations: CS, contaminated and supplemented; CU, contaminated and unsupplemented; n/a, not analysed; UU, uncontaminated and unsupplemented.
*Values in a column in a given trial are significantly different CU vs. CS and CU vs. UU (Dunnett's test).

No mortality or culling were observed during any of the trials. The productive performance was not affected either by the fumonisin contamination or the additive supplementation in any trial.

In all trials, the CU group showed higher Sa/So ratio than the UU group. The supplementation of the diet of sows with the additive at the minimum use level (group CS) reduced the Sa/So ratio after 28 days compared with the CU group. In trial 1, on day 28, the faecal content of FB1 in the CS group was lower than the CU group; the content of HFB1 was higher in the CS group compared to CU group.

3.3.4 | Conclusions on efficacy

The use of the additive at the minimum proposed use level of 60 U/kg in feeds contaminated with fumonisin, resulted in a significant reduction in the Sa/So ratio in five studies in weaned piglets and four in sows. There was also the reduction in the excretion of FB1 and an increase in the excretion of HFB1 which was reported in one study in weaned piglets and one in sows. Therefore, the FEEDAP Panel concludes that the additive is efficacious in reducing the contamination of feed by fumonisins when added at the minimum use level of 60 U/kg in feed for weaned piglets and sows. This conclusion can be extrapolated to all *Suidae*.

[53]Technical dossier/Supplementary information 2023-01-12/Annex Sin 13.

4 | CONCLUSIONS

No viable cells nor DNA of the production strain were detected in the final product. Therefore, the additive does not pose any safety concern regarding the production strain.

The additive is safe up to 60 U/kg complete feed for weaned and suckling piglets and pigs for fattening, and all minor growing porcine species. In the absence of adequate data, the Panel cannot conclude on the safety of the additive at the maximum proposed use level (360 U/kg) nor on the safety for sows and minor reproductive porcine species.

The use of the feed additive in animal nutrition under the proposed conditions of use is safe for the consumers and environment.

The additive is considered to be a respiratory sensitiser; however, the exposure through inhalation is unlikely. The additive is neither a skin nor an eye irritant; no conclusions could be made on the skin sensitisation potential.

The additive is efficacious in reducing the fumonisins contamination in feed when added at the minimum use level of 60 U/kg feed for all *Suidae*.

ABBREVIATIONS

CFU	colony forming unit
EURL	European Union Reference Laboratory
FAO	Food Agricultural Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
LOQ	limit of quantification
OECD	Organisation for Economic Co-operation and Development
RH	relative humidity

ACKNOWLEDGEMENTS

The Panel wishes to thank the following for the support provided to this scientific output: Paul Brantom and Lieve Herman.

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2021-00470

PANEL MEMBERS

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

LEGAL NOTICE

Relevant information or parts of this scientific output have been blackened in accordance with the confidentiality requests formulated by the applicant pending a decision thereon by the European Commission. The full output has been shared with the European Commission, EU Member States and the applicant. The blackening will be subject to review once the decision on the confidentiality requests is adopted by the European Commission.

REFERENCES

Caloni, F., Spotti, M., Pompa, G., Zucco, F., Stammati, A., & De Angelis, I. (2002). Evaluation of fumonisin B1 and its metabolites absorption and toxicity on intestinal cells line Caco-2. *Toxicon*, *40*, 1181–1188.

Collins, T. F., Sprando, R. L., Black, T. N., Olejnik, N., Eppley, R. M., Shackelford, M. E., Howard, P. C., Rorie, J. I., & Bryant, M. (2006). Ruggles DI 2006 effects of aminopentol on in utero development in rats. *Food and Chemical Toxicology*, *44*, 161–169.

EFSA (European Food Safety Authority). (2007). Opinion of the scientific committee on a request from EFSA on the introduction of a qualified presumption of safety (QPS) approach for assessment of selected microorganisms referred to EFSA. *EFSA Journal*, *5*(12), 587. https://doi.org/10.2903/j.efsa.2007.587

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis, K., Allende, A., Álvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., De Cesare, A., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Cocconcelli, P. S., Fernández Escámez, P. S., Prieto Maradona, M., … Herman, L. (2023). Scientific opinion on the update of the list of qualified presumption of safety (QPS) recommended microorganisms intentionally added to food or feed as notified to EFSA. *EFSA Journal*, *21*(1), 7747. https://doi.org/10.2903/j.efsa.2023.7747

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen, H.-K., Barregard, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., Cottrill, B., Dinovi, M., Edler, L., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. R., Nebbia, C. S., Petersen, A., Rose, M., Roudot, A.-C., Schwerdtle, T., Vleminckx, C., Vollmer, G., … Alexander, J. (2018). Scientific opinion on the appropriateness to set a group health-based guidance value for fumonisins and their modified forms. *EFSA Journal*, *16*(2), 5172. https://doi.org/10.2903/j.efsa.2018.5172

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

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2014). Scientific opinion on the safety and efficacy of fumonisin esterase (FUMzyme®) as a technological feed additive for pigs. *EFSA Journal*, *12*(5), 3667. https://doi.org/10.2903/j.efsa.2014.3667

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., Villa, R. E., … López Puente, S. (2016). Scientific opinion on the safety and efficacy of fumonisin esterase (FUMzyme®) as a technological feed additive for all avian species. *EFSA Journal*, *14*(11), 4617. https://doi.org/10.2903/j.efsa.2016.4617

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Innocenti, M. L. (2017a). Guidance on the assessment of the safety of feed additives for the consumer. *EFSA Journal*, *15*(10), 5022. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Innocenti, M. L. (2017b). Guidance on the identity, characterisation and conditions of use of feed additives. *EFSA Journal*, *15*(10), 5023. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Martino, L. (2017c). Guidance on the assessment of the safety of feed additives for the target species. *EFSA Journal*, *15*(10), 5021. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., Lopez Puente, S., Mayo, B., Ramos, F., Saarela, M., Villa, R. E., … Mantovani, A. (2018a). Scientific opinion on the safety and efficacy of fumonisin esterase from Komagataella phaffii DSM 32159 as a technological feed additive for pigs and poultry. *EFSA Journal*, *16*(5), 5269. https://doi.org/10.2903/j.efsa.2018.5269

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Martino, L. (2018b). Guidance on the assessment of the efficacy of feed additives. *EFSA Journal*, *16*(5), 5274. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Galobart, J. (2018c). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*, *16*(3), 5206. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 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, R. E., Woutersen, R., Brock, T., de Knecht, J., … Azimonti, G. (2019). Guidance on the assessment of the safety of feed additives for the environment. *EFSA Journal*, *17*(4), 5648. https://doi.org/10.2903/j.efsa.2019.5648

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances usedin Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Dusemund, B., Kos Durjava, M., Kouba, M., Lopez-Alonso, M., Lopez Puente, S., Marcon, F., Mayo, B., Pechova, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Brozzi, R., … Lopez-Galvez, G. (2020). Scientific opinion on the safety and efficacy of fumonisin esterase from Komagataella phaffii DSM 32159 as a feed additive for all animal species. *EFSA Journal*, *18*(7), 6207. https://doi.org/10.2903/j.efsa.2020.6207

Gelderblom, W. C., Cawood, M. E., Snyman, S. D., Vleggaar, R., & Marasas, W. F. (1993). Structure-activity relationships of fumonisins in short-term carcinogenesis and cytotoxicity assays. *Food and Chemical Toxicology*, *31*, 407–414.

Grenier, B., Bracarense, A. P., Schwartz, H. E., Trumel, C., Cossalter, A. M., Schatzmayr, G., Kolf-Clauw, M., Moll, W. D., & Oswald, I. P. (2012). The low intestinal and hepatic toxicity of hydrolyzed fumonisin B(1) correlates with its inability to alter the metabolism of sphingolipids. *Biochemical Pharmacology*, *83*, 1465–1473.

Gu, M. J., Han, S. E., Hwang, K., Mayer, E., Reisinger, N., Schatzmayr, D., Park, B. C., Han, S. H., & Yun, C. H. (2019). Hydrolyzed fumonisin B1 induces less inflammatory responses than fumonisin B1 in the co-culture model of porcine intestinal epithelial and immune cells. *Toxicology Letters*, *305*, 110–116.

Hahn, I., Nagl, V., Schwartz-Zimmermann, H. E., Varga, E., Schwarz, C., Slavik, V., Reisinger, N., Malachova, A., Cirlini, M., Generotti, S., Dall'Asta, C., Krska, R., Moll, W. D., & Berthiller, F. (2015). Effects of orally administered fumonisin B1 (FB1), partially hydrolysed FB1, hydrolysed FB1 and N-(1-deoxy-D-fructos-1-yl) FB1 on the sphingolipid metabolism in rats. *Food and Chemical Toxicology*, *76*, 11–18.

Howard, P. C., Couch, L. H., Patton, R. E., Eppley, R. M., Doerge, D. R., Churchwell, M. I., Marques, M. M., & Okerberg, C. V. (2002). Comparison of the toxicity of several fumonisin derivatives in a 28-day feeding study with female B6C3F1 mice. *Toxicology and Applied Pharmacology*, *185*, 153–165.

Humpf, H. U., Schmelz, E. M., Meredith, F. I., Vesper, H., Vales, T. R., Wang, E., Menaldino, D. S., Liotta, D. C., & Merrill, A. H., Jr. (1998). Acylation of naturally occurring and synthetic 1-deoxysphinganines by ceramide synthase. Formation of N-palmitoyl-aminopentol produces a toxic metabolite of hydrolyzed fumonisin, AP1, and a new category of ceramide synthase inhibitor. *The Journal of Biological Chemistry*, *273*, 19060–19064.

Russell, J. B., & Forsberg, N. (1986). Production of tricarballylic acid by rumen microorganisms and its potential toxicity in ruminant tissue metabolism. *The British Journal of Nutrition*, *56*, 153–162.

Schwartz, R., Topley, M., & Russell, J. B. (1988). Effect of tricarballylic acid, a nonmetabolizable rumen fermentation product of trans-aconitic acid on Mg, Ca and Zn utilization of rats. *The Journal of Nutrition*, *118*, 6–188.

Seefelder, W., Humpf, H. U., Schwerdt, G., Freudinger, R., & Gekle, M. (2003). Induction of apoptosis in cultured human proximal tubule cells by fumonisins and fumonisin metabolites. *Toxicology and Applied Pharmacology*, *192*(2), 146–153.

Seiferlein, M., Humpf, H. U., Voss, K. A., Sullards, M. C., Allegood, J. C., Wang, E., & Merrill, A. H., Jr. (2007). Hydrolyzed fumonisins HFB1 and HFB2 are acylated in vitro and in vivo by ceramide synthase to form cytotoxic N-acyl-metabolites. *Molecular Nutrition & Food Research*, *51*, 1120–1130.

Sturmberger, L., Chappell, T., Geier, M., Krainer, F., Day, K. J., Vide, U., Trstenjak, S., Schiefer, A., Richardson, T., Soriaga, L., Darnhofer, B., Birner-Gruenberger, R., Glick, B. S., Tolstorukov, I., Cregg, J., Madden, K., & Glieder, A. (2016). Refined Pichia pastoris reference genome sequence. *Journal of Biotechnology*, *235*, 121–131.

Voss, K. A., Riley, R. T., Snook, M. E., & van Gelineau-Waes, J. (2009). Reproductive and sphingolipid metabolic effects of fumonisin B1 and its alkaline hydrolysis product in LM/Bc mice: Hydrolyzed fumonisin B1 did not cause neural tube defects. *Toxicological Sciences*, *112*, 459–467.

How to cite this article: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Dusemund, B., Durjava, M., Kouba, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Villa, R. E., Woutersen, R., Martelli, G., Brozzi, R., … Radovnikovic, A. (2024). Safety and efficacy of a feed additive consisting of fumonisin esterase produced with *Komagataella phaffii* NCAIM (P) Y001485 for all pigs (piglets, pigs for fattening, sows and minor growing and reproductive porcine species) (Dr. Bata Ltd.). *EFSA Journal*, *22*(3), e8614. https://doi.org/10.2903/j.efsa.2024.8614