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Safety and efficacy of fumonisin esterase from *Komagataella phaffii* DSM 32159 as a feed additive for all animal species

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of fumonisin esterase from *Komagataella phaffii* DSM 32159 as a technological feed additive for all animal species. The product has been already assessed by the FEEDAP Panel for use in pigs and poultry feed as technological additive and was granted with an authorisation in the EU for this use. In the current application, the additive is intended to be used in fermenting feeds with the purpose to reduce the contamination of feed by fumonisins. New data provided showed that no recombinant DNA could be detected in three batches of the product. The evidence provided – based on the data submitted in the previous and the current application – allowed the FEEDAP Panel to conclude that the additive is safe for the target animals under the proposed conditions of use. The Panel also considered valid the previous conclusions in which the safety of the additive for consumers and the environment was established. The additive is not toxic by inhalation and the respiratory exposure is likely to be low; however, a risk of sensitisation via the respiratory route cannot be excluded. The additive is non-irritant to skin and eyes and is not considered as a dermal sensitiser. The additive has the capacity to degrade fumonisins in fermenting feed (with a fumonisin content within the guidance limits operating in the EU) when used at the minimum recommended dose of 40 U/kg feed. The FEEDAP Panel notes that the efficacy has been demonstrated only in silages, not in other fermenting feeds like e.g. liquid feeds.

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1. Introduction

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

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

The European Commission received a request from Biomin GmbH² for authorisation of the product fumonisin esterase (FUMzyme®), when used as a feed additive for all animal 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). 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 12 November 2019.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product fumonisin esterase (FUMzyme®), when used under the proposed conditions of use (see Section 3.1).

1.2. Additional information

The product under assessment is an enzyme-based additive, fumonisin esterase, produced by *Komagataella phaffii* DSM 32159 and is intended to degrade fumonisin mycotoxins found as contaminants of feed. The safety and efficacy of this additive for use in pigs and poultry feed were evaluated by the FEEDAP Panel in 2018 (EFSA FEEDAP Panel, 2018a). The safety and efficacy of the same enzyme produced by *Komagataella pastoris* DSM 26643 were the subject of other two opinions of the FEEDAP Panel (EFSA FEEDAP Panel, 2014, 2016).

Fumonisin esterase (EC 3.1.1.87; 1m03) produced by *Komagataella phaffii* (DSM 32159) is currently authorised for use in all pigs and poultry species.³ Fumonisin esterase (EC 3.1.1.87; 1m03) produced by *Komagataella pastoris* (DSM 26643) is currently authorised for use in pigs⁴ and avian species.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 dossier⁶ in support of the authorisation request for the use of fumonisin esterase (FUMzyme®) 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.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the fumonisin esterase (EC 3.1.1.87) (FUMzyme®) in animal feed are valid and applicable for the current application.⁷

¹ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

² Biomin GmbH, Erber Campus 1, 3131 Getzersdorf, Austria.

³ Commission Implementing Regulation (EU) 2018/1568 of 18 October 2018 concerning the authorisation of a preparation of fumonisin esterase produced by *Komagataella phaffii* (DSM 32159) as a feed additive for all pigs and all poultry species. OJ L 262, 19.10.2018, p. 34.

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-2019-0061.

⁷ The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2017-0005_fumzyme.pdf

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of fumonisin esterase (EC 3.1.1.87) (FUMzyme®) 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 identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018c) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The present application concerns an additive containing the enzyme fumonisin esterase intended for use as a technological additive (functional group: substances for reduction of the contamination of feed by mycotoxins) for all animal species. The proposed claim is to reduce the contamination of fumonisin B1 (FB1) and B2 (FB2) in fermenting feeds. The additive is produced by the genetically modified microorganism *Komagataella phaffii* DSM 32159.

3.1. Characterisation and conditions of use

The additive contains by specification a minimum of 3,000 U esterase/g.⁹ The characterisation of this additive, including characterisation of the production strain *K. phaffii* DSM 32159 and the genetic modifications, has been described in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2018a). Equally, the production process of the strain and the manufacturing process of the additive are the same as those in the previous application and assessed by the FEEDAP Panel. Only the new or relevant information concerning the characterisation of the additive is described below.

The applicant provided new data concerning the determination of recombinant DNA of the production strain in the final product.¹⁰ No recombinant DNA could be detected by real-time qPCR in three batches of the product (in triplicate), with a detection limit of 10 ng/g product or 10⁴ cells/g of product.

Considering that when FUMzyme® is applied in feed to be fermented (e.g. silage), it is used as spray application, with the additive dissolved in water, a stability study in water was submitted.¹¹ FUMzyme® was dissolved in aqueous solutions, either water or FCE buffer¹² (average initial activity was 6,367 U/mL in water and 6,604 U/mL in FCE buffer) and stored at room temperature for 48 h; two replicates per medium and three samples per replicate were analysed. The losses reported ranged from 4.4% to 12.9%.

The FEEDAP Panel also concluded in the previous opinion that the use of fumonisin esterase in feed would not interfere with the analytical determination of other structural classes of mycotoxins (EFSA FEEDAP Panel, 2018a).

The additive is intended for use in fermenting feeds for all animal species at a minimum inclusion rate of 40 U/kg fresh material. No maximum incorporation rate is proposed. No withdrawal period is foreseen.

3.2. Safety

3.2.1. Safety aspects related to the production organism

The recipient strain *K. phaffii* used for the production of the enzyme is a species considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment when used for

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 enzymatic activity that releases 1 μmol propane-1,2,3-tricarboxylic acid per minute from 100 IM FB1 in 20 mM Tris-Cl buffer pH 8.0 with 0.1 mg/mL bovine serum albumin at 30°C.

¹⁰ Technical dossier/Supplementary Information March 2020/Annex (i).

¹¹ Technical Dossier/Section II/Annex II-75.

¹² FCE Buffer: 20 mM TrisHCl pH 8, 0.1 mg/mL BSA.

enzyme production (EFSA BIOHAZ Panel, 800). As a result of the genetic modification, the production strain overproduces fumonisin esterase and carries several copies of the Zeocin™ resistance gene and the Geneticin™ resistance gene integrated into its chromosome (EFSA FEEDAP Panel, 2018a). The applicant provided further data that confirmed that neither the viable production strain nor its recombinant DNA were found in three recent batches of the final product.¹⁰ Therefore, the Panel confirmed the conclusions reached in 2018 that the additive fumonisin esterase produced by fermentation with *K. phaffii* DSM 32159 does not give safety concerns deriving from the production strain.

3.2.2. Toxicological studies

The applicant submitted toxicological studies already assessed by the FEEDAP Panel in a previous opinion (EFSA FEEDAP Panel, 2018a).

Three genotoxicity/mutagenicity GLP studies were provided: a bacterial reverse mutation test conducted according to the OECD guideline 471,¹³ an *in vitro* chromosome aberration test conducted according to the OECD guideline 473¹⁴ and an *in vivo* mammalian erythrocyte micronucleus test conducted according to the OECD guideline 474.¹⁵ From the results of these three studies, the FEEDAP Panel concluded that the additive was not mutagenic or clastogenic/aneugenic (EFSA FEEDAP Panel, 2018a).

A subchronic (90-day) oral toxicity good laboratory practice (GLP) study with Wistar rats was conducted with FUMzyme according to the OECD guideline 408.¹⁶ From the results of this study, the FEEDAP Panel concluded that the additive did not exert a sub-chronic toxicity (EFSA FEEDAP Panel, 2018a).

3.2.3. Safety for the target species

Safety concerns from the additive may derive either from the enzyme itself or from the residues of the fermentation process/production strain remaining in the final product. The recipient strain *K. phaffii* used for the production of the enzyme belongs to a species considered by EFSA to be suitable for the QPS approach to assessment of safety, and the final product raised no safety concerns regarding the production strain (Section 3.2.1).

The applicant has provided data showing that the enzyme is fully degraded during the ensiling process (see Section 3.3. Efficacy), and therefore, animals will not be exposed to the additive at time of feeding.

Therefore, in view of the above, the FEEDAP Panel considers that the use of the additive fumonisin esterase produced by fermentation with *K. phaffii* DSM 32159 will not rise safety concerns for the target species when used in fermenting feeds.

To further support the safety of the product for the target animals, the applicant provided the same tolerance studies and the 90-day toxicity study already assessed in the previous opinion (EFSA FEEDAP Panel, 2018a; see also Section 3.2.1). From the latter, the applicant proposed to derive the maximum safe level in feed.

In the previous opinion, the FEEDAP Panel concluded that the proposed maximum application rate of 300 U/kg feed was safe in all poultry species and pig species. This conclusion was based on the results of tolerance studies in weaned piglets, chickens for fattening, turkeys for fattening and laying hens, in which animals tolerated 100-fold this maximum application rate. The FEEDAP Panel considers that these conclusions would apply to the present application which further support the safety for the target animals.

From the results in the 90-day toxicity study already assessed in the previous opinion, a no observed adverse effect level (NOAEL) of 2,000 mg test item/kg body weight (bw) (the highest dose tested) was identified (test item used fumonisin esterase at a concentration of 8,650 U/g).¹⁷ The maximum safe daily dose for the target species was derived following the EFSA Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b); the calculation included the application of an uncertainty factor (UF) of 100 to the NOAEL. The results showed that the lowest maximum safe additive concentration in feed would be 1,927 U/kg feed for chickens for fattening, thus, supporting the safety of the product for all animal species under the proposed conditions of use.

¹³ Technical Dossier/Section III/Annex III-34.

¹⁴ Technical Dossier/Section III/Annex III-35.

¹⁵ Technical Dossier/Section III/Annex III-36.

¹⁶ Technical Dossier/Section III/Annex III-37.

¹⁷ Annex III_32 CoA Fumzyme activity_PIB001T_new.

3.2.3.1. Conclusions on safety for the target species

The FEEDAP Panel concludes that the additive is safe for the target animals under the proposed conditions of use.

3.2.4. Safety for the consumer, the user and the environment

The safety of FUMzyme® for the consumer has been assessed in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2018a). In that opinion the Panel concluded that 'The use of the additive in pigs and in poultry under the conditions specified will not introduce hazards for consumers'. This conclusion was based on the fact that the recipient strain qualifies for the QPS approach to safety assessment, on the absence of viable cells and DNA in the final product, on the results of the genotoxicity and sub-chronic oral toxicity studies and on the reduced toxicity of the metabolites resulting from the complete or partial de-esterification of fumonisins. The FEEDAP Panel considers that the same conclusions would apply to the current application and the proposed new use in fermenting feeds will not introduce any hazards not already considered in the previous assessment. Therefore, the use of FUMzyme® under the proposed conditions of use is considered safe for the consumer.

The production organism is considered suitable for the QPS approach to safety assessment. Taking into account the nature of the genetic modification and the extensive purification undertaken to exclude DNA fragments from the final additive, the production or retention of toxic metabolites produced during fermentation is considered improbable. This was confirmed by the results of two *in vitro* and one *in vivo* tests for genotoxicity and a subchronic oral toxicity study. No evidence of mutagenicity or genotoxicity was detected and no evidence of toxicity found in the oral toxicity study.

Metabolites resulting from the complete or partial de-esterification of fumonisins have been previously assessed for safety and found to be less toxic than the parent mycotoxin. The action of fumonisin esterase on any contaminating fumonisins will be independent of source and essentially the same in pig and poultry digestive tracts and will act to reduce any toxic load. Consequently, the use of the additive in pigs and poultry will not introduce hazards for consumers.

The safety of the additive for the user has been assessed in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2018a). The Panel concluded that 'The additive is not toxic by inhalation and the respiratory exposure is likely to be low; however, a risk of sensitisation via the respiratory route cannot be excluded. The additive is non-irritant to skin and eyes and is not considered as a dermal sensitiser'. These conclusions are valid and applicable to the current application.

The safety of the additive for the environment has been assessed in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2018a). The FEEDAP Panel based its assessment on the safety of the production strain and on the likely degradation of the enzyme in the digestive tract of the animal and in soils, concluding that no risks for the environment were expected following the use of the additive in feeds under the proposed conditions of use. These conclusions are valid and applicable to the current application for the use of the additive in fermenting feed of all animal species.

3.3. Efficacy

The applicant claims that FUMzyme® exerts its function in fermenting feeds (e.g. silages), and thus the additive has the capacity to reduce the contamination of fumonisin B1 (FB1) and B2 (FB2) in fermenting feeds.

To support the efficacy of the additive the applicant submitted three *in vitro* studies.

The *in vitro* studies were conducted using silage (shredded whole maize crop or maize grains) either artificially or naturally contaminated with fumonisins at different levels. The additive was tested at three different dosages (20, 30 and 40 U/kg feed), both alone and combination with an acid-based premixture or a microbial premixture usually added to silage to enhance fermentation.

3.3.1. *In vitro* studies

3.3.1.1. Study 1

A 90-day laboratory-scale silage trial was performed with shredded whole crop maize at full-ripe stage (dry matter content of 39.8 % and soluble carbohydrate content of 2.04 % fresh matter (FM)), artificially contaminated with FB1 (2,280 µg/kg).¹⁸ A fumonisin-contaminated control group was

¹⁸ Technical Dossier/Section IV/Annex IV_01, IV_02, IV_03, IV_04.

compared to a fumonisin-contaminated treatment group, treated with the additive at 20 U/Kg FM. Six 1.8-L buckets (containing about 0.5 kg fresh material) and six 5.8-L buckets (containing about 2 kg fresh material) were prepared for both, the control and the treatment groups. Small sample silos were used for the analysis on day 2 and 7 after ensiling, while the larger buckets were used for analysis on day 44 and 90 of the trial. Uniform density was achieved by closely monitoring the packing pressure, which was 3 bar for small sample silos and 6 bar for large sample silos. FB1 concentration (assessed at day 2, 7 and 90 after ensiling) and fermentation parameters (pH, dry matter, selected carbohydrates and organic acids, assessed at day 2, 7, 44 and 90 after ensiling) were monitored in the study. Fumonisin esterase activity was assessed in one treated sample on day 7.¹⁹

Statistical analysis was done with non-parametric comparisons (Mann–Whitney U-tests) of the group data due to low replicate numbers.

A decrease of FB1 concentration was observed already in the control group; however, the inclusion of the additive significantly improved the reduction process at every sampling point ($p \leq 0.05$). FUMzyme® significantly reduced FB1 until day 7, which is consistent with the additive's complete degradation, registered on day 7. The results of the first trial are summed up in Table 1.

Table 1: Fumonisin B1 (FB1, $\mu\text{g}/\text{kg}$ fresh matter) concentration in artificially contaminated silage without FUMzyme® (control group) or with FUMzyme® (treatment group) in the study 1. At day 0, the silage contained 2,280 μg FB1/kg kg fresh matter

Day	Control	Treatment
2	2,801 ^a	1,768 ^b
7	1,687 ^a	326 ^b
90	900 ^a	266 ^b

a,b: For a given row, different superscripts indicate significant differences ($p \leq 0.05$).

3.3.1.2. Study 2

A 7-day laboratory-scale silage trial was performed with shredded maize grains (dry matter content of 79 % and soluble carbohydrate content of 0.9 % FM), artificially contaminated with FB1 (5,686 $\mu\text{g}/\text{kg}$) and FB2 (1,389 $\mu\text{g}/\text{kg}$).²⁰ A fumonisin-contaminated feed material without the additive (control group) was compared to a fumonisin-contaminated feed material treated with the additive at 30 U/kg fresh matter (treatment group). Fifteen 1.8-L buckets (containing about 0.7 kg of fresh material) were prepared for each group. Uniform density was achieved by closely monitoring the packing pressure, which was 3 bar. Fumonisin concentration, fermentation parameters (pH, dry matter and sugar content, organic acid composition) and fumonisin esterase activity were assessed at day 3, 5 and 7 after ensiling.

Statistical analysis was performed with either parametric or non-parametric comparisons of the group data. For the efficacy test within the trial (FUM vs FUM_FZ), the choice of test (T-tests with or without correction for variance equality or U-tests) was made according to normality of data and homogeneity of variances.

FB1 and FB2 concentrations were already reduced at day 3 in both control and treatment groups. However, the reduction was more pronounced in samples treated with the additive and proceeded until the end of the trial. In the control group, FB1 and FB2 concentration did not further decrease after day 3. As regards fumonisin esterase activity, its monitoring showed a decrease over the experiment reaching the maximum (about 30 % of the initial measurement) at day 7. A summary of the main results is reported in Table 2.

¹⁹ Technical Dossier/Supplementary Information/March 2020.

²⁰ Technical Dossier/Section IV/Annex IV_05, IV_06, IV_07, IV_08, IV_09.

Table 2: Fumonisin B1 (FB1, µg/kg fresh matter) and B2 (FB2, µg/kg fresh matter) concentration in artificially contaminated silage without FUMzyme® (control group) or with FUMzyme® (treatment group) in the study 2. At day 0 the silage contained 5,686 µg FB2/kg fresh matter and 1,389 µg FB1/kg fresh matter

Day	Parameter	Control	Treatment
3	FB1	3,630 ^a	349 ^b
	FB2	698 ^a	118 ^b
5	FB1	4,377 ^a	130 ^b
	FB2	756 ^a	66 ^b
7	FB1	3,918 ^a	200 ^b
	FB2	872 ^a	175 ^b

a,b: For a given row, different superscripts indicate significant differences ($p \leq 0.05$).

In parallel, a compatibility trial with three additional treatments was performed to evaluate the compatibility of the additive (at 30 U/kg) with acid-based premixtures used for silage treatment. In this study, three groups were used: (i) a control feed consisting on a fumonisin-contaminated feed material treated with an acid-based premixture²¹ (control group), (ii) the control feed treated consecutively with FUMzyme® (treatment group_cons) and (iii) the control feed treated simultaneously with FUMzyme® (treatment group_sim). Initial contamination of FB1 was 5,686 µg/kg and of FB2 1,389 µg/kg. FB1 and FB2 concentrations, fermentation parameters (pH, dry matter and sugar content, organic acid composition) as well as fumonisin esterase activity were assessed at day 3, 5 and 7 after ensiling. For the compatibility test, either analysis of variance (ANOVA) with post hoc Tukey HSD test, Welch ANOVA with post hoc Tamhane T2 test or Kruskal–Wallis test with pairwise Dunn-tests as follow-up were run, depending on normality of data and homogeneity of variances.

FB1 and FB2 concentration were reduced at day 3 in both control and treatment groups. However, the reduction was more pronounced in presence of the additive with respect to the control. Concentration of FB1 and FB2 were significantly lower in all treated groups with respect to the control at all sampling times. Moreover, FB1 and FB2 concentration remained stable in the control group after day 3, while they further decreased in both treatment groups, with no statistically significant difference emerging between them. The main results are reported in Table 3.

Fumonisin esterase activity was significantly reduced when applied simultaneously to the acid based premixture: at day 0 it was reduced by 61% and from day 3 to 7 by 80%; conversely, only a slight reduction was detected when the additive was added after the acid based premixture. As regards fermentation parameters, statistically significant differences were registered for some of them during the trial. However, they were not consistent and did not follow a specific trend. As reported for the other trials, significant differences in fermentation parameters were not consistent.

Table 3: Fumonisin B1 (FB1, µg/kg fresh matter) and B2 (FB2, µg/kg fresh matter) concentration in artificially contaminated silage treated with an acid-based premixture (control group), consecutively treated with FUMzyme® (treatment group_cons) or simultaneously treated with FUMzyme® (treatment group_sim) in study 2-compatibility trial. At day 0, the silage contained 5,686 µg FB1/kg fresh matter and 1,389 µg FB2/kg fresh matter

Day	Parameter	Control	Treatment group_cons	Treatment group_sim
3	FB1	3,439 ^a	826 ^b	716 ^b
	FB2	595 ^a	211 ^b	248 ^b
5	FB1	3,888 ^a	217 ^b	287 ^b
	FB2	759 ^a	72 ^b	150 ^b
7	FB1	3,973 ^a	294 ^b	375 ^b
	FB2	1,004 ^a	120 ^b	210 ^b

a,b: For a given row, different superscripts indicate significant differences ($p \leq 0.05$).

²¹ The acid-based premixture (containing a preparation of sodium benzoate, propionic acid and sodium propionate, 620 g/kg) had a pH-value of 5.0 and was applied at a dose of 0.25% in silage.

3.3.1.3. Study 3

A 90-day laboratory-scale silage trial was performed with shredded maize grains (dry matter content of 75% and soluble carbohydrate content of 2.7%), naturally contaminated with FB1 (1,207 µg/kg) and FB2 (232 µg/kg).²² A fumonisin-contaminated control group was compared to a fumonisin-contaminated treatment group treated with the additive at 40 U/kg fresh matter. Fifteen 1.8-L buckets (holding about 0.7 kg of fresh material) were prepared for each group. Uniform density was achieved by closely monitoring the packing pressure, which was 3 bar. Both groups were supplemented with a microbial silage premixture²³ to improve the fermentation process. FB1 and FB2 concentrations as well as fermentation parameters (pH, dry matter and sugar content, organic acid composition) and fumonisin esterase activity were assessed at day 6, 30, 60 and 90 after ensiling.

Statistical analysis was performed with either parametric or non-parametric comparisons of the group data. The choice of test (T-tests with or without correction for variance equality or U-tests) was made according to normality of data and homogeneity of variances.

FB1 and FB2 concentrations were reduced in both control and treated groups by day 6; these reductions were more pronounced in the treated group ($p = 0.003$). The reduction did not proceed further in the control group, while it remained under the limit of quantification (LOQ, 50 µg/kg) throughout the trial in the treated group. A summary of the results is reported in Table 4.

Table 4: Fumonisin B1 (FB1, µg/kg fresh matter) and B2 (FB2, µg/Kg fresh matter) concentration in naturally contaminated silage without FUMzyme® (control group) and with FUMzyme® (treatment group) supplemented with a microbial premixture in study 3. At day 0, the silage contained 1,207 µg FB1/kg kg fresh matter and 232 µg FB2/kg kg fresh matter

Day	Parameter	Control	Treatment
6	FB1	453 ^a	10 ^b
	FB2	145 ^a	13 ^b
30	FB1	769 ^a	22 ^b
	FB2	168 ^a	10 ^b
60	FB1	618 ^a	10 ^b
	FB2	199 ^a	10 ^b
90	FB1	558 ^a	10 ^b
	FB2	135 ^a	10 ^b

a,b: For a given row, different superscripts indicate significant differences ($p \leq 0.05$).

The additive decreased during the study: already at day 6 showed a decrease to 27.1 %, at day 6 the reduction was to about 11 % and it was not detectable at the end of the trial.

Although the efficacy has been shown against FB1 and FB2, as already expressed in its previous opinion, the FEEDAP Panel considers that the enzyme will be equally effective in its action against all recognised fumonisins (EFSA FEEDAP Panel, 2018a).

3.3.2. Conclusions on efficacy

The additive has the capacity to degrade fumonisins in fermenting feed (with a fumonisin content within the guidance limits operating in the EU)²⁴ when used at the minimum recommended dose of 40 U/kg feed. This conclusion is based on the results from three studies in which statistically significant fumonisins reduction was recorded in treated silage. The FEEDAP Panel notes that the efficacy has been demonstrated only in silages, not in other fermenting feeds like e.g. liquid feeds.

4. Conclusions

The FEEDAP Panel concludes that the use of additive under the proposed conditions of use is safe for target animals, consumers and the environment.

²² Technical Dossier/Section IV/Annex IV_10, IV_11, IV_12, IV_13.

²³ The microbial premixture consisted of *Lactobacillus plantarum*, *Lactobacillus brevis* and *Lactobacillus kefir*. It was applied at a dose of 1×10^5 CFU/g in silage.

²⁴ Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. OJ L 229, 23.8.2006, p. 7.

The additive is not toxic by inhalation and the respiratory exposure is likely to be low; however, a risk of sensitisation via the respiratory route cannot be excluded. The additive is non-irritant to skin and eyes and is not considered as a dermal sensitiser.

The additive has the capacity to degrade fumonisins in fermenting feed (with a fumonisin content within the guidance limits operating in the EU) when used at the minimum recommended dose of 40 U/kg feed. This conclusion is based on the results from three studies in which statistically significant fumonisins reduction was recorded in treated silage. The FEEDAP Panel notes that the efficacy has been demonstrated only in silages, not in other fermenting feeds like e.g. liquid feeds.

5. Documentation as provided to EFSA/Chronology

Date	Event
19/09/2019	Dossier received by EFSA. FUMzyme® (fumonisin esterase) for all animal species. Submitted by Biomin GmbH
30/09/2019	Reception mandate from the European Commission
12/11/2019	Application validated by EFSA – Start of the scientific assessment
11/12/2019	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, Efficacy</i>
12/02/2020	Comments received from Member States
02/03/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
01/07/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ANOVA	analysis of variance
BIOHAZ	EFSA Scientific Panel on Biological Hazards
bw	body weight
CFU	colony forming unit
DM	dry matter
EURL	European Union Reference Laboratory
FB1	fumonisin B1
FB2	fumonisin B2
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FM	fresh matter
GLP	good laboratory practice
LOQ	limit of quantification
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
QPS	Qualified Presumption of Safety
UF	uncertainty Factor