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SCIENTIFIC OPINION

Safety of the feed additive consisting of endo-1,4-β-xylanase (produced with *Trichoderma reesei* CBS 143953), subtilisin (produced with *Bacillus subtilis* CBS 143946) and α-amylase (produced with *Bacillus amyloliquefaciens* CBS 143954) (Avizyme® 1505) for all poultry species (Danisco (UK) Ltd.)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety of the feed additive consisting of endo-1,4-β-xylanase (produced with *Trichoderma reesei* CBS 143953), subtilisin (produced with *Bacillus subtilis* CBS 143946) and α-amylase (produced with *Bacillus amyloliquefaciens* CBS 143954) (Avizyme® 1505) as a zootechnical feed additive for all poultry species. The additive is authorised in feed for chickens and turkeys for fattening, ducks and laying hens. In 2020, the FEEDAP Panel issued an opinion for the renewal of the authorisation of the additive for the species/categories for which there is an authorisation, a reduction of the minimum recommended level in turkeys for fattening and the extension of use to all poultry species. In that assessment, the Panel could not conclude on the safety of the additive due to uncertainties on the characterisation of the production strains and the possible presence of their viable cells and DNA in the final product. Moreover, limitations were identified in the xylanase specifications and xylanase method of analysis. The applicant submitted information to address the limitations previously identified. The Panel concluded that the additive is safe for the target species under the proposed conditions of use. The use of Avizyme® 1505 in animal nutrition is considered safe for the consumer and the environment. The additive is a mild irritant to skin and eyes; it is not a dermal sensitiser but should be considered a respiratory sensitiser. The additive is efficacious in ducks at 75 U endo-1,4-β-xylanase, 1000 U subtilisin and 100 U α-amylase/kg of complete feed. In other poultry species for fattening (including turkeys), reared for breeding and reared for laying, the additive is efficacious at 187.5 U endo-1,4-β-xylanase, 2500 U subtilisin and 250 U α-amylase per kg of complete feed and at 300 U endo-1,4-β-xylanase, 4000 U subtilisin and 400 U α-amylase per kg of complete feed for all poultry species for laying (except for ducks).

KEYWORDS

Avizyme® 1505, digestibility enhancers, poultry, safety, zootechnical additives

CONTENTS

1 | INTRODUCTION

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

Regulation (EC) No 1831/2003 establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Danisco (UK) Ltd, trading as Danisco Animal Nutrition and represented by Genencor International B.V., is seeking a Community authorisation of endo-1,4-beta-xylanase (EC 3.2.1.8) derived from *Trichoderma reesei*, alpha-amylase (EC 3.2.1.1) derived from *Bacillus amyloliquefaciens* and protease (EC 3.4.21.62) derived from *Bacillus subtillis* as a feed additive to be used as a digestibility enhancer for all poultry species. (Table 1).

TABLE 1 Description of the substances.

Category of additive	Zootechnical additives
Functional group of additive	Digestibility additives
Description	endo-1,4-beta-xylanase (EC 3.2.1.8) derived from *Trichoderma reesei*, alpha-amylase (EC 3.2.1.1) derived from *Bacillus amyloliquefaciens* and protease (EC 3.4.21.62) derived from *Bacillus subtilis*
Target animal category	All ducks and all other avian species (excluding breeders)
Applicant	Danisco (UK) Ltd, trading as Danisco Animal Nutrition and represented by Genencor International B.V.
Type of request	New opinion

On 30 January 2020, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of the European Food Safety Authority (EFSA), in its opinion on the safety and efficacy of the product, could not conclude on the safety because uncertainty remains in the characterisation of the production strains and the possible presence in the final product of viable production strains and their DNA, of endo-1,4-beta-xylanase (EC 3.2.1.8) derived from *Trichoderma reesei*, alpha-amylase (EC 3.2.1.1) derived from *Bacillus amyloliquefaciens* and protease (EC 3.4.21.62) derived from *Bacillus subtilis* in all poultry species.

The Commission gave the possibility to the applicant to submit supplementary information and data in order to complete the assessment and to allow a revision of the EFSA's opinion. The new data have been received on 03 March 2021.

In view of the above, the Commission asks the Authority to deliver a new opinion on endo-1,4-beta-xylanase (EC 3.2.1.8) derived from *Trichoderma reesei*, alpha-amylase (EC 3.2.1.1) derived from *Bacillus amyloliquefaciens* and protease (EC 3.4.21.62) derived from *Bacillus subtilis* as a feed additive for all poultry species based on the additional data submitted by the applicant, in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002.

1.2 | Additional information

The additive, with the trade name Avizyme® 1505, is a preparation of endo-1,4-beta-xylanase produced with a genetically modified strain of *Trichoderma reesei* (ATCC PTA 5588), subtilisin produced with a genetically modified strain of *Bacillus subtilis* (ATCC 2107) and alpha-amylase produced with a genetically modified strain of *Bacillus amyloliquefaciens* (ATCC 3978).

The additive is authorised (4a10) to be used in feed for chickens for fattening, ducks, turkeys for fattening[1] and laying hens.[2]

EFSA adopted several opinions on the safety and efficacy of this additive (EFSA, 2009a, 2009b; EFSA FEEDAP Panel, 2011, 2020).

[1]Commission Regulation (EC) No 1087/2009 of 12 November 2009 concerning the authorisation of an enzyme preparation of endo-1,4-beta-xylanase produced by *Trichoderma reesei* (ATCC PTA 5588), subtilisin produced by *Bacillus subtilis* (ATCC 2107) and alpha-amylase produced by *Bacillus amyloliquefaciens* (ATCC 3978) as a feed additive for chickens for fattening, for ducks and for turkeys for fattening (holder of authorisation Danisco Animal Nutrition, legal entity Finnfeeds International Limited). OJ L 297, 13.11.2009. p. 4.

[2]Commission Implementing Regulation (EU) No 389/2011 of 19 April 2011 concerning the authorisation of an enzyme preparation of endo-1,4-beta-xylanase, sutilisin and alpha-amylase as a feed additive for laying hens (holder of authorisation Danisco Animal Nutrition). OJ L 104, 19.4.2011. p. 7.

2 | DATA AND METHODOLOGIES

2.1 | Data

The present assessment is based on data submitted by the applicant in the form of supplementary information[3] to a previous application on the same product.[4] The dossier was received on 1/3/2021 and the general information and supporting documentation are available on Open.EFSA at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00694.

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of endo-1,4-beta-xylanase (produced with *T. reesei* CBS 143953), subtilisin (produced with *B. subtilis* CBS 143946) and alpha-amylase (produced with *B. amyloliquefaciens* CBS 143954) (Avizyme® 1505) is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: 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, 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), Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021), EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (EFSA, 2021) and Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023).

3 | ASSESSMENT

The additive Avizyme® 1505 is a preparation of endo-1,4-beta-xylanase produced with a genetically modified strain of *T. reesei* (CBS 143953 (formerly deposited at the American Type Culture Collection (ATCC) PTA 5588); xylanase), subtilisin produced with a genetically modified strain of *B. subtilis* (CBS 143946 (formerly ATCC 2107); subtilisin, protease) and alpha-amylase produced with a genetically modified strain of *B. amyloliquefaciens* (CBS 143954 (formerly ATCC 3978); amylase).[6] The additive is authorised at the minimum guaranteed enzyme activity of 1500 U xylanase/g, 20,000 U subtilisin/g and 2000 U amylase/g. The composition of the additive and the production strains were first characterised and described by the FEEDAP Panel in 2009 (EFSA, 2009a, 2009b).

The additive is authorised as a zootechnical additive (functional group: digestibility enhancers) at the inclusion levels of 187.5 U xylanase,[7] 2500 U subtilisin[8] and 250 U amylase[9] per kg of complete feed for chickens for fattening; 75 U xylanase, 1000 U subtilisin and 100 U amylase per kg of complete feed for ducks and 300 U xylanase, 4000 U subtilisin and 400 U amylase per kg of complete feed for turkeys for fattening and laying hens.

In the last opinion on this product (EFSA FEEDAP Panel, 2020), the FEEDAP Panel identified uncertainties in the characterisation of the production strains and the possible presence of viable cells of the production strains and their DNA in the final product. Consequently, the Panel considered that the information provided by the applicant did not fulfil the minimum requirements to support that the additive remains safe under the approved conditions of use for the target species, consumers, users and environment. The same conclusions applied also to the new target species for which a request for an extension of use was made. Moreover, limitations were identified in the analysis method of xylanase and in the xylanase specifications of the additive.

The applicant has now provided supplementary information regarding the characterisation of the production strains, presence of viable cells and recombinant DNA in the additive and clarifications on the xylanase activity present in the additive.

[3]Dossier reference: EFSA-Q-2021-00694.

[4]Dossier reference: FAD-2018-0084.

[5]Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[6]Annex_II_19_Strain_deposit_certificates.

[7]1 U of endo-1,4-β-xylanase is the amount of enzyme that liberates 0.5 μmol of reducing sugar (xylose equivalents) per minute from a cross-linked oat spelt xylan at pH 5.3 and 50°C.

[8]One subtilisin (protease) unit is defined as the amount of enzyme, which liberates 2.3 micrograms of phenolic compound (expressed as tyrosine equivalents), per minute from a casein substrate at pH 10.0 and 50°C.

[9]One alpha-amylase unit is defined as the amount of enzyme, which liberates 1 micromole of glucosidic linkages, per minute from a water insoluble cross-linked starch polymer substrate at pH 6.5 and 37 °C.

3.1 | Characterisation

3.1.1 | Characterisation of the production organisms

The applicant did not declare any new genetic modifications in the production strains of the endo-1,4-beta-xylanase, subtilisin and alpha-amylase. In the context of the current submission, the applicant submitted new data, reported below, to address the data gaps related to the characterisation of the three genetically modified production strains.

3.1.1.1 | *Trichoderma reesei CBS 143953 – Endo-1,4-beta-xylanase production strain*

The endo-1,4-beta-xylanase present in the additive is produced with *T. reesei* CBS 143953, which contains [redacted] The assessment of the genetic modification of the production strain was performed in a previous evaluation (EFSA, 2009b) and the Panel concluded that the genetic modification of the xylanase production strain does not raise any safety concern.

In its previous application, the applicant submitted updated data confirming the taxonomic identification of the recipient strain of CBS 143953 as *T. reesei* (EFSA FEEDAP Panel, 2020).

Although some *Trichoderma* species are known to be capable of producing a variety of mycotoxins and antifungal metabolites, no data addressing the capacity of the production strain to produce mycotoxins was provided in the context of the 2020 assessment. In the current submission, the applicant submitted data on the ability of an intermediate strain ([redacted]), used in the development of CBS 143953, to produce trichothecenes (trichodermin, trichodermol and harzianum A) or gliotoxin.[10] The results showed that neither trichothecenes nor gliotoxin was detected in the supernatant of cultures of the intermediate strain tested. *T. reesei* seems to be unable to produce mycotoxins (EFSA, 2007; EFSA BIOHAZ Panel, 2020; Frisvad et al., 2018), but it is known to produce peptaibols, such as paracelsin A, C and D (Frisvad et al., 2018), and its genome has been shown to harbour genes for two peptaibol synthases (Kubicek et al., 2007). Those peptaibols are peptides with antimicrobial activity and are typically produced under stressful conditions (Frisvad et al., 2018). Peptaibol production predominantly occurs in solid fermentation and correlates with conidiation (Kubicek et al., 2007; Tisch & Schmoll, 2010).

The FEEDAP Panel considers that the lack of antimicrobial activity in the additive under assessment demonstrated in the 2020 opinion (EFSA FEEDAP Panel, 2020) would indicate that if peptaibols are produced under the fermentation conditions, their concentration would be of no concern and that safety concerns derived from the presence of other secondary metabolites potentially produced by *T. reesei* CBS 143953 would be addressed by the toxicological studies already assessed in 2009 (EFSA, 2009b).

3.1.1.2 | *Bacillus subtilis CBS 143946 – Subtilisin production strain*

The subtilisin present in the additive is produced with *B. subtilis* CBS 143946, which contains a [redacted]. The assessment of the genetic modification of the production strain was performed in a previous assessment (EFSA, 2009b) and the Panel confirmed in its conclusion that the production strain carries a [redacted].

In its previous opinion of 2020, the FEEDAP Panel noted that uncertainties remained on the identification and characterisation of *B. subtilis* CBS 143946, including the presence of antimicrobial resistance genes ([redacted]) and its toxigenic potential. The applicant submitted new data to characterise the subtilisin production strain.

The taxonomic identification of CBS 143946 as *B. subtilis* was confirmed by phylogenomic analysis using the 446 most conserved core proteins across public available genomes.[11] The analysis grouped CBS 143946 with *B. subtilis* type strain DSM 10^T.

The whole genome sequence (WGS) data of the production strain *B. subtilis* CBS 143946 were interrogated for the presence of antimicrobial resistance (AMR) genes by a search against the CARD and ResFinder databases.[12] No hits of concern above the thresholds set by EFSA (EFSA, 2021) were identified except for [redacted].

The toxigenic potential of *B. subtilis* CBS 143946 was assessed according to the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a).[13] No lysis of Vero cells was detected, so *B. subtilis* CBS 143946 is considered to be not toxigenic.

3.1.1.3 | *Bacillus amyloliquefaciens CBS 143954 – Alpha-amylase production strain*

The alpha-amylase present in the additive is produced with *B. amyloliquefaciens* CBS 143954, which contains an alpha-amylase encoding gene from *B. amyloliquefaciens* and [redacted]. The assessment

[10]Annex_1a_T reesei secondary metabolites and Annex_1b_Metabolite profile.
[11]Annex_2_taxonomic analysis_B subtilis.
[12]Annex_7_ CBS 143946 _Antibiotic resistance.
[13]Annex_12_CBS 143946_cytotox report.

of the genetic modification of the production strain was performed in a previous assessment (EFSA, 2009b) and the Panel confirmed in its conclusion that the production strain carries [redacted].

In its previous opinion of 2020, the FEEDAP Panel noted that uncertainties remained on the identification and characterisation of *B. amyloliquefaciens* CBS 143954, including the presence of antimicrobial resistance genes ([redacted]). The applicant submitted new data to characterise the alpha-amylase production strain.

The taxonomic identification of CBS 143954 as *B. amyloliquefaciens* was confirmed by phylogenomic analysis using the 465 most conserved core proteins across public available genomes.[14] The analysis grouped CBS 143954 with *B. amyloliquefaciens* type strain DSM 7^T.

The WGS data of the production strain *B. amyloliquefaciens* CBS 143954 were interrogated for the presence of AMR genes by a search against the CARD and ResFinder databases.[15] The search showed hits having identity and coverage above the thresholds set by EFSA (EFSA, 2021): [redacted]. The interrogation also identified a putative *clbA* gene which may confer resistance to chloramphenicol. However, the antimicrobial susceptibility profile of the strain was evaluated in 2020 (EFSA FEEDAP Panel, 2020) and showed that the strain was [redacted].

3.1.1.4 | *Presence of viable cells and DNA of the production strains in the final product*

In the previous assessment, uncertainties remained regarding the presence of viable cells and DNA of the three production strains in the additive (EFSA FEEDAP Panel, 2020).

The applicant submitted new data to exclude the presence of viable cells of the three strains in three batches of the liquid intermediate products (194,284 U [redacted]/g [183,901–204,621 U [redacted]/g]; 211,913 U [redacted]/g [176,038–254,565 U [redacted]/g]; 47,605 U [redacted]/g [47,241–47,804 U [redacted]/g]) used to formulate the additive.[16]

The batches were analysed in triplicate. [redacted] yeast extract glucose chloramphenicol (YGC) agar plates and incubated at 30°C for 120 h ([redacted]), or on tryptic soy agar plates (TSA) and incubated at 37°C for 48 h ([redacted]). [redacted] TSA supplemented with chloramphenicol and incubated at 37°C for 48 h ([redacted]). [redacted]. Colonies were detected in two of the batches tested but were not identified as belonging to the production strain based on strain-specific PCR analysis. Therefore, it can be concluded that no viable cells of any of the production strains were detected in intermediate products used to formulate the additive.

The applicant also provided data to exclude the presence of DNA of the three production strains in three batches of xylanase and subtilisin intermediate products (194,704 U xylanase/g (179,905–205,193 U xylanase/g) and 246,708 U subtilisin/g (190,661–340,563 U subtilisin/g), respectively) and in three batches of the amylase solid concentrate (24,175 U amylase/g (22,881–25,806 U amylase/g)).[17] The three batches described above were subject to PCR analysis, in triplicate (1 mL or 1 g sample). The primers targeted [redacted] from *T. reesei* CBS 143953, [redacted] from *B. subtilis* CBS 143946 or [redacted] from *B. amyloliquefaciens* CBS 143954, [redacted]. The protocol included a lysis step. The limit of detection in samples spiked with DNA was ≤ 10 ng/mL. The analysis showed no amplification in the samples.

3.1.2 | Manufacturing process

The applicant did not declare any modification on the manufacturing process of the endo-1,4-beta-xylanase or of the alpha-amylase. A modification introduced in the manufacturing process of the subtilisin is described below.

3.1.2.1 | *Manufacturing process of the subtilisin*

In the context of the current submission, the applicant declared a change in the manufacturing process of the subtilisin compared to the previous assessment (EFSA FEEDAP Panel, 2020). [redacted]. No other modifications were declared. In addition, the applicant provided information on [redacted].[18] [redacted]

[14]Annex_8_taxonomic analysis_B amyloliquefaciens.
[15]Annex_8_CBS 143954_Antibiotic resistance.
[16]Annex_1_production strain analysis and Annex_2_process flow.
[17]Annex_10a_rDNA_xylanase, Annex_10b_rDNA_amylase and Annex_10c_rDNA_protease.
[18]Response_SIn100311_EFSA-Q-2021_Avizyme 1505.

[redacted].[19] [redacted]. In the current submission, the applicant indicated the identification of the strains and provided some information on the origin of the strains and steps followed for their development. Data were also submitted to exclude the presence of [redacted] in the product.[20] Three batches of the [redacted] were tested in triplicate. [redacted] on YGC agar and plates were incubated for 120 h at 30°C. [redacted]. Colonies were detected in the batches of [redacted], but they were not identified as belonging to the production strain. Therefore, it can be concluded that no viable cells of any of the [redacted] production strains were detected.

3.1.3 | Characterisation of the additive

No changes have been declared in the composition or physical properties of the final formulation of the additive with respect to the one authorised.

In view of the changes proposed in the manufacturing process of the subtilisin, the applicant submitted new data on the batch-to-batch variation.

Avizyme® 1505 is authorised as a powder form with a minimum guaranteed enzyme activity of 1500 U xylanase/g, 20,000 U subtilisin[21]/g and 2000 U amylase[22]/g. The batch-to-batch variation was studied in five recent batches obtained [redacted] in the subtilisin production.[23] The mean enzyme activities were 5389 U/g for xylanase (range 4040–5994 U/g), 27,162 U/g for subtilisin (range 22,315–30,530 U/g) and 7504 U/g for amylase (same value in all batches tested).[24] These data are in line with the batch-to-batch variation reported in the previous assessment (EFSA FEEDAP Panel, 2020).

In the context of 2020 opinion, the applicant proposed a new definition for the units of xylanase which was different than the one described in the authorisation Regulations ('one xylanase unit is the amount of enzyme that releases 0.48 µmol of reducing sugar equivalents (expressed as xylose by the DNS reducing sugar method) from wheat arabinoxylan per min at pH 4.2 and 50°C' versus '1 U of endo-1,4-β-xylanase is the amount of enzyme that liberates 0.5 µmol of reducing sugar (xylose equivalents) per minute from a cross-linked oat spelt xylan at pH 5.3 and 50°C'). Although the new definition was according to the method used for the control of the additive evaluated in the context of another application (FAD-2010-0007), the applicability of such method in the context of the Avizyme® 1505 application could not be assessed due to the lack of data. Therefore, the Panel could not establish the correspondence between the new and previous xylanase activity definition.

In the context of the current assessment, the applicant explained that the new xylanase unit definition was established following the calculation of a conversion factor (0.48 (µmol/min)/xylanase U) between the authorised and the newly proposed method.[25] The correspondence of the new definition/method of analysis with the previous one was confirmed by the results of the batch-to-batch variation described above, which showed that the xylanase activities measured with the new method are compliant with the specifications set in the authorisation (minimum guaranteed xylanase activity of 1500 U/g). Moreover, the European Union Reference Laboratory (EURL) evaluated the newly proposed method by the applicant to analyse the xylanase activity and considered the difference of the two definitions in terms of the measured values very small (in the range of 5%).

The FEEDAP Panel concludes that the newly submitted information allows to consider the proposed definition of xylanase unit valid and applicable for the product under assessment.

3.1.4 | Conditions of use

The additive is currently authorised for use in feed at the minimum use level of:

- 187.5 U xylanase, 2500 U subtilisin and 250 U amylase/kg of complete feed for chickens for fattening,
- 75 U xylanase, 1000 U subtilisin and 100 U amylase/kg of complete feed for ducks,
- 300 U xylanase, 4000 U subtilisin and 400 U amylase/kg of complete feed for turkeys for fattening and laying hens.

The applicant did not request any changes in the current conditions of authorisation except for turkeys for fattening, for which has asked to reduce the minimum recommended level to 187.5 U xylanase, 2500 U subtilisin and 250 U amylase/kg feed.

[19] Annex_4_enzyme activity.

[20] Annex_1_production strain analysis, Annex 1_viable cells report and Annex_2_manu process.

[21] One subtilisin (protease) unit is defined as the amount of enzyme, which liberates 2.3 micrograms of phenolic compound (expressed as tyrosine equivalents), per minute from a casein substrate at pH 10.0 and 50°C.

[22] One alpha-amylase unit is defined as the amount of enzyme, which liberates 1 micromole of glucosidic linkages, per minute from a water insoluble cross-linked starch polymer substrate at pH 6.5 and 37°C.

[23] Annex_11_batch_to_batch_CoA.

[24] Annex II 4 and response_SIn141222_EFSA-Q-2021_00694_Avizyme 1505.

[25] Annex_4_unit definition data, Complementary info_ EFSA_Q_2018_0084_Avizyme1505_01March2021 and response_SIn150224_EFSA-Q-2021-00694_Avizyme 1505.

In addition, the applicant proposed the extension of use as follows:

- 187.5 U xylanase, 2500 U subtilisin and 250 U amylase/kg of complete feed for all poultry species for fattening, reared for breeding and reared for laying (except for ducks)
- 300 U xylanase, 4000 U subtilisin and 400 U amylase/kg of complete feed for all poultry species for laying (except for ducks).

3.2 | Safety

In the previous assessments (EFSA, 2009a, 2009b; EFSA FEEDAP Panel, 2011) and based on the tolerance studies and toxicological data submitted, the FEEDAP Panel concluded that the use of Avizyme® 1505 as a feed additive was safe for the target species, the consumers of products derived from animals fed with the additive and for the environment. The Panel also concluded that the additive is a mild irritant to skin and eyes; it is not a dermal sensitiser but should be considered a potential respiratory sensitiser.

In the context of the 2020 assessment, the applicant conducted a literature search to provide evidence that in the light of the current knowledge the additive remained safe under the approved conditions. However, considering the uncertainties in the characterisation of the production strains and the possible presence of their viable cells and DNA in the final product, the FEEDAP Panel could not confirm the previously drawn conclusions regarding the safety of the additive for the target species, consumers, users and environment. This conclusion applied also to the new target species for which a request for an extension of use was made (all poultry species for fattening, reared for breeding and reared for laying and all poultry species for laying).

The data made available in the current assessment (see Section 3.1.1) allowed to properly characterise the production strains *T. reesei* CBS 143953, *B. subtilis* CBS 143946 and *B. amyloliquefaciens* CBS 143954 and exclude the presence of their viable cells and DNA in intermediate products used to formulate the additive. Although *B. subtilis* CBS 143946 and *B. amyloliquefaciens* CBS 143954 contain AMR genes [redacted], there is no risk identified since neither viable cells nor DNA was detected in the product. Therefore, the production of Avizyme® 1505 does not raise safety concerns as regards the genetically modified production strains used in the manufacturing of the enzymes present in the additive.

In view of the above, the FEEDAP Panel concludes that Avizyme® 1505 is safe for chickens for fattening, ducks, turkeys for fattening and laying hens under the proposed conditions of use. The conclusion can be extended to all poultry species for fattening, reared for breeding, reared for laying and all poultry species for laying.

Avizyme® 1505 is safe for the consumers and the proposed extension of use of the additive to the new species/categories would not introduce risks not already evaluated in the previous opinions.

Regarding the safety for the user, the additive is a mild irritant to skin and eyes; it is not a dermal sensitiser but should be considered a respiratory sensitiser.

No risks to the environment are expected and no further environmental risk assessment is required. The proposed use of the additive to the new species/categories would not introduce risks not already evaluated in previous opinions.

3.3 | Efficacy

The product is authorised in ducks at 75 U xylanase, 1000 U subtilisin and 100 U amylase/kg of complete feed, in turkeys for fattening and laying hens at 300 U xylanase, 4000 U subtilisin and 400 U amylase per kg of complete feed and in chickens for fattening at 187.5 U xylanase, 2500 U subtilisin and 250 U amylase per kg of complete feed.

In its last assessment (EFSA FEEDAP Panel, 2020), the Panel concluded that the additive is efficacious in ducks at 75 U xylanase, 1000 U subtilisin and 100 U amylase/kg of complete feed, in turkeys for fattening, all poultry species for fattening, reared for breeding and reared for laying (except for ducks) at 187.5 U xylanase, 2500 U subtilisin and 250 U amylase per kg of complete feed and at 300 U xylanase, 4000 U subtilisin and 400 U amylase per kg of complete feed for all poultry species for laying (except for ducks).

In the context of the current assessment, the applicant provided evidence that the proposed definition of xylanase unit is valid and applicable for the product under assessment. Therefore, considering that the composition and conditions of use of the additive have not been modified, the Panel concludes that the additive is efficacious in ducks at 75 U xylanase, 1000 U subtilisin and 100 U amylase/kg of complete feed, in all poultry species for fattening (including turkeys), reared for breeding and reared for laying (except for ducks) at 187.5 U xylanase, 2500 U subtilisin and 250 U amylase per kg of complete feed and at 300 U xylanase, 4000 U subtilisin and 400 U amylase per kg of complete feed for all poultry species for laying (except for ducks).

3.4 | Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[26] and good manufacturing practice.

4 | CONCLUSION

No viable cells and DNA of the production strains *Trichoderma reesei* CBS 143953, *Bacillus subtilis* CBS 143946 and *Bacillus amyloliquefaciens* CBS 143954 were detected in intermediate products used to formulate the additive. Moreover, viable cells of the [redacted] production strains [redacted] were not detected. Therefore, the production of Avizyme® 1505 does not raise safety concerns as regards the production strains used in the manufacturing of the enzymes present in the additive.

Avizyme® 1505 is safe for chickens for fattening, ducks, turkeys for fattening and laying hens under the proposed conditions of use. The conclusion can be extended to all poultry species for fattening, reared for breeding, reared for laying and all poultry species for laying.

The use of Avizyme® 1505 in animal nutrition is considered safe for the consumer and the environment.

Regarding the safety for the user, the additive is a mild irritant to skin and eyes; it is not a dermal sensitiser but should be considered a respiratory sensitiser.

The Panel concludes that the additive is efficacious in ducks at 75 U xylanase, 1000 U subtilisin and 100 U amylase/kg of complete feed, in turkeys for fattening, all poultry species for fattening, reared for breeding and reared for laying (except for ducks) at 187.5 U xylanase, 2500 U subtilisin and 250 U amylase per kg of complete feed and at 300 U xylanase, 4000 U subtilisin and 400 U amylase per kg of complete feed for all poultry species for laying (except for ducks).

ABBREVIATIONS

FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
QPS	Qualified Presumption of Safety
TSA	tryptic soy agar plates
WGS	whole genome sequence

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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.

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[26]Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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