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Assessment of the feed additive consisting of alpha-galactosidase produced by *Saccharomyces cerevisiae* CBS 615.94 and endo-1,4-beta-glucanase produced by *Aspergillus niger* CBS 120604 (Agal-Pro BL/BL-L®) for use in chickens for fattening, minor poultry species for fattening and chickens reared for laying for the renewal of its authorisation (Kerry Ingredients & Flavours Ltd.)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the assessment of the application for renewal of Agal-Pro BL/BL-L®. The additive is a preparation of alpha-galactosidase produced by a genetically modified strain of *Saccharomyces cerevisiae* (CBS 615.94) and endo-1,4-beta-glucanase produced by a non-genetically modified strain of *Aspergillus niger* (CBS 120604). It is intended to be used as a zootechnical additive (functional group: digestibility enhancers) in chickens for fattening in its solid and liquid forms (Agal-Pro BL® and Agal-Pro BL-L®), and in minor poultry species for fattening and chickens reared for laying only in its solid form (Agal-Pro BL®). The applicant has provided evidence that the additive currently on the market complies with the existing conditions of authorisation. There is no new evidence that would lead the FEEDAP Panel to reconsider its previous conclusions. Thus, the Panel concludes that the additive remains safe for the target animals, consumers and the environment under the authorised conditions of use. Regarding user safety, the additive is considered an irritant to the skin and eyes and a dermal sensitiser. Due to the proteinaceous nature of the active substances, it should be considered a respiratory sensitiser. There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

Keywords: zootechnical additives, digestibility enhancers, alpha-galactosidase, endo-1,4-beta-glucanase, *Saccharomyces cerevisiae* CBS 615.94, *Aspergillus niger* CBS 120604, safety

Requestor: European Commission

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest 1 year before the expiry date of the authorisation.

The European Commission received a request from Kerry Ingredients & Flavours Ltd.[2] for the renewal of the authorisation of the additive consisting of alpha-galactosidase (produced by *Saccharomyces cerevisiae* CBS 615.94) and endo-1,4-betaglucanase (produced by *Aspergillus niger* CBS 120604) (Agal-Pro BL/BL-L®), when used as a feed additive for chickens for fattening, minor poultry species for fattening and chickens reared for laying (category: zootechnical additive; functional group: digestibility enhancer).

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 14(1) (renewal of the authorisation). The dossier was received on 10 February 2021 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00128. The particulars and documents in support of the application were considered valid by EFSA as of 22 March 2022.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the feed additive consisting of alpha-galactosidase (produced by *Saccharomyces cerevisiae* CBS 615.94) and endo-1,4-betaglucanase (produced by *Aspergillus niger* CBS 120604) (Agal-Pro BL/BL-L®), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive contains alpha-galactosidase produced by a genetically modified strain of *Saccharomyces cerevisiae* (CBS 615.94) and endo-1,4-betaglucanase produced by a non-genetically modified strain of *Aspergillus niger* (CBS 120604). The trade name of the additive is Agal-Pro® and is available in solid (Agal-Pro BL®) and liquid (Agal-Pro BL-L®) forms.

EFSA issued two opinions on the safety and efficacy of the solid form of the additive when used in chickens for fattening (EFSA FEEDAP Panel, 2011) and in chickens reared for laying and minor poultry species for fattening (EFSA FEEDAP Panel, 2013), one opinion on its liquid form when used in chickens for fattening (EFSA FEEDAP Panel, 2014) and one opinion on both liquid and solid forms when used in laying hens and minor poultry species for laying (EFSA FEEDAP Panel, 2015).

The additive is authorised in the European Union (EU) as a zootechnical additive (functional group: digestibility enhancer) (4a17) in its solid and liquid forms for use in chickens for fattening,[3] and in its solid form for use in minor poultry species for fattening and chickens reared for laying[4] and laying hens and minor poultry species for laying.[5]

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Kerry Ingretiens & Flavours Ltd., Kilnagleary, Carrigaline PA43A597, County Cock, Ireland.
[3] Commission Implementing Regulation (EU) 2015/1104 of 8 July 2015 amending Implementing Regulation (EU) No 237/2012 of 19 March 2012 as regards a new form of alpha-galactosidase (EC 3.2.1.22) produced by *Saccharomyces cerevisiae* (CBS 615.94) and endo-1,4-beta-glucanase (EC 3.2.1.4) produced by *Aspergillus niger* (CBS 120604) as a feed additive for chickens for fattening (holder of authorisation Kerry Ingredients and Flavours). OJ L 80, 20.3.2012. pp. 1–4.
[4] Commission Implementing Regulation (EU) No 1365/2013 of 18 December 2013 concerning the authorisation of a preparation of alpha-galactosidase produced by *Saccharomyces cerevisiae* (CBS 615.94) and endo-1,4-beta-glucanase produced by *Aspergillus niger* (CBS 120604) as a feed additive for minor poultry species for fattening and for chickens reared for laying (holder of authorisation Kerry Ingredients and Flavours). OJ L 343, 19.12.2013. pp. 29–31.
[5] Commission Implementing Regulation (EU) No 2015/2382 of 17 December 2015 concerning the authorisation of the preparation of alpha-galactosidase (EC 3.2.1.22) produced by *Saccharomyces cerevisiae* (CBS 615.94) and endo-1,4-beta-glucanase (EC 3.2.1.4) produced by *Aspergillus niger* (CBS 120604) as a feed additive for laying hens and minor poultry species for laying (holder of the authorisation Kerry Ingredients and Flavours). OJ L 332, 18.12.2015. pp. 54–56.

The applicant has requested the renewal of the authorisation of the additive for chickens for fattening in both the solid and liquid forms, and for minor poultry species for fattening and chickens reared for laying in the solid form.

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 additive consisting of alpha-galactosidase produced by the genetically modified *S. cerevisiae* CBS 615.94 and endo-1,4-betaglucanase produced by *A. niger* CBS 120604 (Agal-Pro BL/BL-L®) as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' elicitation knowledge, to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.[7]

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 the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021).

3. Assessment

The present assessment regards the renewal of the authorisation of the product containing alpha-galactosidase (Enzyme Commission Number 3.2.1.22) produced by a genetically modified strain of *S. cerevisiae* CBS 615.94 and endo-1,4-beta-glucanase (Enzyme Commission Number 3.2.1.4) produced by *A. niger* CBS 120604 (trade name: Agal-Pro BL/BL-L®). It is intended to be used in chickens for fattening in its solid and liquid forms (Agal-Pro BL® and Agal-Pro BL-L®), and in minor poultry species for fattening and chickens reared for laying only in its solid form (Agal-Pro BL®).

3.1. Characterisation

3.1.1. Characterisation of the production microorganisms

3.1.1.1. *Aspergillus niger* CBS 120604

The strain *A. niger* is deposited in the Westerdijk Fungal Biodiversity Institute culture collection (CBS-KNAW) under the accession number CBS 120604.[9] It has not been genetically modified.[10]

The genome of the production strain *A. niger* was sequenced and used for identification purposes. The taxonomic identification was confirmed by [redacted][11] The identification was further confirmed by analysis of ITS and of partial sequences of the calmodulin (*CaM*), β-tubulin (*BenA*) and RNA polymerase II (*RPB2*) genes following the methodology described in Samson et al. (2014).[12]

[6] FEED dossier reference: FAD-2021-0010.
[7] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/system/files/2013-02/FinRep-FAD-2009-0014.pdf
[8] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
[9] Annex II.2.1.2.Q.RN CBS 120604- Deposit Declaration 2019.
[10] Annex II.2.1.2.R.RN ZS non GMO declaration.
[11] Annex II.1.4.Z.a. RN Aspergillus niger ZS_WGS_analysis.
[12] Annex II.2.1.2.T.RN Identification ZS_2017 Westerfijk.

The applicant investigated the capacity of the production strain *A. niger* to produce antimicrobials in four samples resulting from the fermentation process.[13] The analysis was conducted using a disc-diffusion agar method against the following reference strains: *Bacillus cereus* ATCC 11778, *Bacillus circulans* ATCC 4516, *Serratia marcescens* ATCC 14041, *Streptococcus pyrogens* ATCC 12344, *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 11229. No antimicrobial activity was detected.

The whole genome sequence (WGS) data of the production strain was interrogated for the presence of biosynthetic gene clusters involved in the production of secondary metabolites using antiSMASH (similarity threshold > 50%).[11] No clusters coding for compounds of foreseeable concern were detected.

3.1.1.2. *Saccharomyces cerevisiae* CBS 615.94

The strain *S. cerevisiae* is deposited in the Westerdijk Fungal Biodiversity Institute culture collection (CBS-KNAW) under the accession number CBS 615.94.[14] It has been genetically modified.

The genome of the production strain *S. cerevisiae* was sequenced and used for identification purposes. The taxonomic identification was confirmed [redacted].[15]

Characterisation of the parental or recipient microorganism

The parental/recipient strain is *S. cerevisiae* [redacted], also known as *S. cerevisiae* [redacted], a strain that has not been previously genetically modified. The *S. cerevisiae* [redacted] strain is a [redacted] mutant derived from strain [redacted], which is a [redacted] derived from the wild type strain [redacted].[16]

Description of the genetic modification

The purpose of the genetic modification was to produce the enzyme alpha-galactosidase. The genetically modified strain was derived by introduction of the [redacted] in the genome of the parental strain. The [redacted] reference sequence is [redacted] long and it includes the yeast endogenous sequences [redacted] and [redacted], a stretch of undefined nucleotides (Ns) at the start, the *S. cerevisiae* [redacted] gene [redacted] which complements the [redacted] mutation of the host cell; and it harbours the alpha-galactosidase gene [redacted] from guar [redacted], generating a [redacted] aa protein, in addition to a complete [redacted] gene [redacted] and a truncated [redacted] gene [redacted].

The applicant has provided new data on the characterisation of the genetic modifications following the new guidance documents (EFSA FEEDAP Panel, 2018; EFSA, 2021). The genome of the production strain *S. cerevisiae* CBS 615.94 was analysed by alignment with that of the parental/recipient strain *S. cerevisiae* [redacted]. The analysis demonstrated the insertion of multiple copies (about [redacted] copies) of the [redacted] in the genome of *S. cerevisiae* CBS 615.94 and identified a single integration site in the genome located on chromosome [redacted]. Analysis of the alpha-galactosidase coding sequence in the production strain suggests that the nucleotide sequences of all gene copies are identical. No further modifications have been done to the strain in comparison to those previously described (EFSA FEEDAP Panel, 2011).[17]

3.1.2. Characterisation of the additive

The additive is authorised in two different formulations, a solid powder form (Agal-Pro BL®, referred to as BL) and a liquid form (Agal-Pro BL-L®, referred to as BL-L). The BL form guarantees a

[13] Annex II.1.4.F-1.RN 007-_KERRY-720_ZS S2007133 Antimicrobial Siliker - Method and Results and Annex II.1.4.F-2.RN 010-_KERRY-820_ZS 52008092_93_94 Antimicrobial Siliker.
[14] Annex II.2.1.2.A.RN CBS 615.94-Deposit Declaration 2019.
[15] Annex II.2.1.2.N.RN v2.
[16] Sect-II_Identity_ BL_BL-L - renewal Feb22.
[17] Annex II.2.1.2.O.RN BaseClear 128966_EFSA_ScerevisiaeNS470 GM analysis.

minimum enzymatic activity of 1,000 U alpha-galactosidase[18]/g additive and 5,700 U endo-1,4-beta-glucanase[19]/g additive. The BL-L form guarantees a minimum enzymatic activity of 500 U alpha-galactosidase/g additive and 2,850 U endo-1,4-beta-glucanase/g additive. The solid formulation contains wheat flour (75–80%) as carrier; the liquid formulation contains glycerol (40–50%), sodium benzoate (0.2%) and potassium sorbate (0.1%). The applicant states that the manufacturing process and the composition have not been modified since the authorisation was granted.[16]

Five recent batches of each form of the additive were tested for mean enzyme activity. The BL form showed: 1,142 U alpha-galactosidase/g additive (range: 1,100–1,200 U alpha-galactosidase/g additive) and 6,474 U endo-1,4-beta-glucanase/g additive (range: 5,986–7,015 U endo-1,4-beta-glucanase/g additive). The BL-L form showed 618 U alpha-galactosidase/ g additive (range: 594–636 U alpha-galactosidase/g additive) and 3,197 U endo-1,4-beta-glucanase/g additive (range: 2,945–3,500 U endo-1,4-beta-glucanase/g additive). These measured enzyme activities showed compliance with the specifications set in the authorisation.[20] The applicant provided an indication of the total organic solids (TOS) content of the additive.[21]

The same five batches of each form of the additive were analysed for microbial contamination. *Escherichia coli* and *Salmonella* spp. were not detected in 25 g; total viable counts and coliforms were < 10 CFU/g additive; yeast and filamentous fungi ranged 10–65 CFU/g additive (mean value 31 CFU/g additive) in the solid form and 10–30 CFU/g additive (mean value 17 CFU/g additive) in the liquid form. No data on the presence of *Enterobacteriaceae* were provided.

Three batches of each form of the additive were analysed for arsenic, cadmium, mercury and lead. The three batches of the solid form[22] analysed showed values below the respective limits of quantification (LOQ) of the analytical methods, except for cadmium that showed values ranging 0.018–0.024 mg/kg and for arsenic that showed a value of 0.049 mg/kg in one batch. The three batches of the liquid form[23] showed levels below the respective LOQs of the analytical methods.[24]

Three independent samples resulting from the fermentation process of the production strain *A. niger* CBS 120604 were additionally analysed for mycotoxins including Aflatoxins B1, B2, G1 and G2, deoxynivalenol, fumonisin B1 and B2, ochratoxin A and zearalenone concentration.[25] All the batches showed levels below the respective LOQs of the analytical methods.[26]

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

The presence of viable cells of the production strains was investigated in triplicate in three independent samples resulting from the fermentation process of the production strain *A. niger* and three independent samples resulting from the fermentation process of the production strain *S. cerevisiae*.[27] For each batch, 10 mL sample was diluted in 90 mL 1% peptone solution and filtered through a 0.45 μm

[18] One enzyme unit (EU) is defined as the amount of enzyme that will produce one micromole of para-nitrophenol per minute from p-nitrophenyl-alpha-galactopyranoside (pNPG) under the specified conditions (pH = 5.0; temperature 37°C).

[19] One enzyme unit (EU) is defined as the amount of enzyme that will produce one milligram of reducing sugar (glucose equivalent) from hydrolyses of beta-D-glucans under the specified conditions (pH = 5.0; temperature 50°C).

[20] Annex II.1.3.A.RN: Analysis of five production batches on the amount of active substances.

[21] FAD-2021-0010_Slninfo_Kerry reply_Annex 7June23 and Subsidiary CoA to Annex II.1.3.A.RN.

[22] Annex II.1.5.C Agal Pro BL S2204040 heavy metals 13.4.2022, Annex II.1.5.C1 COA 20267823 AGal-Pro BL batch 0006093401 Heavy metals, Annex II.1.5.D Agal Pro BL 20267823 #6145623 12.8.2022 Metal Testing, Annex II.1.5.D1 COA 20267823 AGal-Pro BL batch 0006146523 Heavy metals, Annex II.1.5.E Agal Pro BL 20267823 #6145636 12.8.2022 Metal Testing and Annex II.1.5.E1 COA 20267823 AGal-Pro BL batch 0006145636 Heavy metals.

[23] Annex II.1.5.F Agal Pro BL L S2202015 heavy metals, Annex II.1.5.F1 COA 20306191 AGal-Pro BL-L batch 0006098851S2202015, Annex II.1.5.G Agal Pro BL L 20306191 #6182713 12.8.2022 Metal Testing, Annex II.1.5.G1 COA 20306191 AGal-Pro BL-L batch 0006182713 S2208065, Annex II.1.5.H Agal Pro BL L 20306.191 #6283334 25.8.2022 Metal Testing and Annex II.1.5.H1 COA 20306191 AGal-Pro BL-L batch 0006283334S2208128.

[24] LOQ: cadmium 0.010 mg/kg, lead 0.050 mg/kg, arsenic 0.040 mg/kg and mercury 0.005 mg/kg.

[25] Annex II.1.4.J.RN ZS AT_HM TLR Report 1066281 S2004131, Annex II.1.4.K.RN ZS TBS Batch 52008093 AT_HM TLR Report 1102728 S2008093 v2 and Annex II.1.4.L.RN ZS TBS Batch 52008092 AT_HM TLR Report 1102727 S2008082 v2.

[26] LOQ: Aflatoxin total < 1.5 μg/kg, deoxynivalenol <100 μg/kg, fumonisin B1 < 5.0 μg/kg, fumonisin B2 < 5.0 μg/kg, ochratoxin A < 1.0 μg/kg and zearalenone < 5.0 μg/kg.

[27] Annexes II.1.4.M.RN NS 20105363 Batch 0003753682 CoA Absence of producer organism Jan22, Annexes II.1.4.N.RN NS 20105363 Batch 0003817439 CoA Absence of producer organism Jan22, Annexes II.1.4.O.RN NS 20105363 Batch 0003875714 CoA Absence of producer organism Jan22, Annexes II.1.4.P.RN ZS 20105469 Batch 0003789078 CoA Absence of producer organism Jan22, Annexes II.1.4.Q.RN ZS 20105469 Batch 52008093 CoA Absence of producer organism Jan22 and Annexes II.1.4.R.RN ZS 20105469 Batch 52008092 CoA Absence of producer organism Jan2.

filter. After the filtration step, the absorbent filter was impregnated with non-selective medium and incubated at 30°C for 5 days.[28] No growth was detected. A positive control was included.

The presence of recombinant DNA from the production strain *S. cerevisiae* CBS 615.94 was tested in three batches of the most concentrated formulation of the additive (Agal-Pro BL), each one tested in triplicate.[29] The starting material was 1 g, primers targeted a strain-specific sequence spanning the insertion cassette with an amplicon size of [redacted] bp, which is smaller than the [redacted] gene present in the strain [redacted]. DNA was extracted and a mechanical lysis step using glass beads was included. Positive and negative controls were included. The limit of detection (LOD) of samples spiked with total genomic DNA of the production strain was 10 ng/g of additive.[30] No DNA of the production strain was detected.

3.1.3. Physical properties of the additive

The applicant has provided new data on the dusting potential of the solid formulation of the additive. It was determined in three batches and using the Stauber–Heubach method; the results showed an average of 515 mg/m^3 (range 479–566 mg/m^3).[31]

No other new data regarding the physico-chemical properties or stability of the additive have been provided. Since no changes have been introduced in the additive or its manufacturing process, the data described in previous opinions still apply.

3.1.4. Conditions of use

The additive is currently authorised for use in feed for:

- Chickens for fattening in its solid and liquid forms at a minimum content of 50 U alpha-galactosidase and 285 U endo-1,4-beta-glucanase per kg of complete feedingstuffs with a moisture content of 12%.
- Minor poultry species for fattening and chickens reared for laying in its solid form at a minimum content of 50 U alpha-galactosidase and 285 U endo-1,4-beta-glucanase per kg of complete feedingstuffs with a moisture content of 12%.

The authorisation under other provisions foresees:

- In the directions for use of the additive and premixture, indicate the storage temperature, storage life and stability to pelleting in chickens for fattening; and indicate the storage conditions and stability to pelleting in minor poultry species for fattening and chickens reared for laying.
- Maximum recommended dose: 100 U alpha-galactosidase/kg of complete feed; 570 U endo-1,4-beta-glucanase/kg of complete feed.
- For safety: breathing protection, glasses and gloves shall be used during handling.

The applicant has requested to maintain the same conditions of use.

3.2. Safety

The applicant states that no adverse effects on target animal, consumer, user or the environment have been reported for the additive since its authorisation.[32]

In its previous opinions (EFSA, 2011; EFSA FEEDAP Panel, 2013, 2014), the FEEDAP Panel concluded that the additive is safe for chickens for fattening, minor poultry species for fattening and chickens reared for laying as well as for the consumers and the environment. Concerning user safety, the Panel concluded that the additive is a skin and eye irritant and a dermal sensitiser and should be considered a respiratory sensitiser.

In the previous opinion (EFSA, 2011) the applicant provided an *in vitro* chromosomal aberration test (OECD Guideline 473) which allows studying the potential of the fermentation product produced

[28] Annex II.1.4.S.RN v2 Report Absence of producer organism in three batches of additives Jan22 and Annex II.1.4.G.RN SAM0301 Screening for Producer organism NS and ZS.
[29] Annex II.1.4.Y.RN COAs 20267823 AGal-Pro BL batches Baseclear Absence DNA analysis v2 Jan22.
[30] Annex II.1.4.X.RN BaseClear 129806 Absence of DNA from ScerevisiaseNS470.
[31] Annex II.4.3.C.RN Dusting Potential AGal-Pro BL Report #21-DSS-1345 Kerry Stauber-Heubach Dec21 and Annex II.4.3.D.RN AGal-Pro BL CoAs S21110620_621_622 Dusting potential.
[32] Sect-III_Safety_AGal-Pro BL _BL-L.

by *A. niger* CBS 120604 to induce structural chromosomal aberrations but not for numerical aberrations. In the current application, a new *in vitro* micronucleus test was submitted with the aim to cover also potential numerical chromosome aberrations.[33] The test item used was an intermediate concentrate resulting from the fermentation process of *A. niger* CBS 120604. The enzymatic activity for endo-1,4-beta-glucanase in the test item is 18,564 U/mL, which is higher than the minimum activity guarantee for the solid and liquid forms of the final product (5,700 and 1,850 U/g of additive, respectively). The FEEDAP Panel considers the test item as representative of the final forms of the additive. The *in vitro* micronucleus test was performed in Chinese Hamster Ovary cells (CHO-K1) according to OECD TG 487 and claimed to be good laboratory practices (GLP) compliant. Based on the results of a dose range finding study, the cells were treated with the test item at 313, 625 and 1,250 μg TOS/mL, corresponding to endo-1,4-beta-glucanase activity of 30.59, 61.07 and 122.16 U/mL in a short treatment (4 + 20 h of recovery) in the absence and presence of metabolic activation. A continuous treatment (24 + 0 h of recovery) without metabolic activation was also applied and cells were treated with 25, 50 and 100 μg TOS/mL, corresponding to an activity of endo-1,4-beta-glucanase ranging from 2.44 to 9.77 U/mL. Cytotoxicity up to 47% was induced by the test item. The frequency of micronuclei in binucleated cells was comparable between treated and negative control cultures. The Panel concludes that the test item did not induce structural and numerical chromosome aberrations under the experimental conditions applied in this study. No additional studies were submitted for the fermentation product containing the alpha-galactosidase. The species *S. cerevisiae* is considered by EFSA to be eligible for the qualified presumption of safety (QPS) approach (EFSA, 2007; EFSA BIOHAZ Panel, 2023). In the view of the FEEDAP Panel, the identity of the strain CBS 615.94 has been established as *S. cerevisiae* and the genetic modifications do not give rise to safety concerns from the toxicological point of view. Consequently, no additional studies are considered needed.

The applicant conducted an extensive literature search (ELS) to provide an updated appraisal on the safety of the additive (target animals, consumers and users) covering the period 2009–2020. It comprised the databases PubMed and SciFinder. The search terms (comprehensive list of search terms considering the target animals, enzymes, safety and adverse effects), the search strategy, the inclusion and exclusion criteria, the description of the review process, and the references selected in the review were provided.[34] In total 188 publications were retrieved and no relevant new information on the safety of the additive for the target species, consumers or users could be detected. No specific information was submitted regarding the safety for the environment. In the previous opinions, the FEEDAP Panel considered that, since the two enzymes are proteins and will be degraded/inactivated during the passage through the digestive tract of animals, no risks for the environment are expected. The Panel is not aware of any information that would lead to modify its previous conclusions. Therefore, the additive is considered to be safe for the environment.

There is no new evidence that would lead the FEEDAP Panel to reconsider its previous conclusions; the additive is considered to remain safe for the target species, consumers and environment. The additive in either form is a skin and eye irritant and a dermal sensitiser and should be considered a respiratory sensitiser.

3.3. Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the conditions of the original authorisations that would have an impact on the efficacy of the additive. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

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[35] and Good Manufacturing Practice.

[33] Annex III.39 RN G22070_ ZS_ in vitro MN Final Study Report target activity betamannanase and Annex III.40 RN Enzyme ZS #5825094 COA- Test material in vitro Micronucleus study G22079 v4.

[34] Sect-III_Safety_AGal-Pro BL _BL-L, Annex III.2.RN Literature review report v3 and FAD-2021-0010_Slninfo_Kerry reply_Annex 27Mar23.

[35] 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.

4. Conclusions

The applicant has provided evidence that the additive currently on the market complies with the existing terms of authorisation.

The Panel concludes that the additive Agal-Pro BL/BL-L® remains safe for the animal species in subject of the current application, consumers and the environment under the authorised conditions of use. Regarding user safety, the additive is considered irritant to skin and eyes and a dermal sensitiser. Due to the proteinaceous nature of the active substances, it should be considered a respiratory sensitiser.

There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

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Abbreviations

BUSCO	Benchmarking Universal Single-Copy Orthologs
CFU	colony forming unit

EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
LOQ	limit of quantification
WGS	whole genome sequence