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Safety and efficacy of a feed additive consisting of β-mannanase produced by *Aspergillus niger* CBS 120604 (Nutrixtend Optim) for use in all poultry for fattening (Kerry Ingredients & Flavours Ltd)

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Abstract

Following a request from the European Commission, the EFSA 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 a product containing β-mannanase produced by a non-genetically modified strain of *Aspergillus niger* (CBS 120604). The commercial name is Nutrixtend Optim and it is intended to be used as a zootechnical feed additive for all poultry for fattening. Based on a tolerance trial in chickens for fattening and the no observed adverse effect level identified in a subchronic oral toxicity study in rats, the additive was considered safe for all poultry for fattening. The Panel concluded that the use of the product as a feed additive does not give rise to concerns for consumers and the environment. The additive is considered an irritant to skin and eyes and a dermal sensitiser. Due to the proteinaceous nature of the active substance, it is also considered a respiratory sensitiser. The Panel concludes that the additive has the potential to be efficacious as a zootechnical additive at the level of inclusion in feed of chickens for fattening of 30 U β-mannanase/kg complete feed. This conclusion was extrapolated to all poultry for fattening.

Keywords: zootechnical additives, digestibility enhancers, β-Mannanase, safety, efficacy, poultry for fattening

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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 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Kerry Ingredients & Flavours Ltd[2] for the authorisation of the additive consisting of β-mannanase produced by *Aspergillus niger* CBS 120604 (Nutrixtend Optim), when used as a feed additive for all poultry for fattening (category: zootechnical additive; functional group: digestibility enhancers).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 18 January 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 β-mannanase produced by *Aspergillus niger* CBS 120604 (Nutrixtend Optim), when used under the proposed conditions of use (see **Section 3.1.6**).

1.2. Additional information

The additive contains β-mannanase produced by *Aspergillus niger* CBS 120604 (Nutrixtend Optim). It has not been previously authorised as a feed additive in the European Union.

EFSA has published four opinions on another feed additive currently authorised (4a17) containing an endo-1,4-β-glucanase produced by the same production strain (EFSA FEEDAP Panel, 2011, 2013, 2014, 2015).

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 in support of the authorisation request for the use of β-mannanase produced by *Aspergillus niger* CBS 120604 (Nutrixtend Optim) as a feed additive.[3]

The dossier was received on 15 June 2021, and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00549.

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.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the endo-1,4-β-mannanase in animal feed.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of β-mannanase produced by *A. niger* CBS 120604 (Nutrixtend Optim) is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance on studies concerning

[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] Kilnagleary (Carrigaline), P43 A597, Co. Cork (Ireland).
[3] FEED dossier reference: FAD-2021-0053.
[4] Evaluation report received on 4 January 2022 and available on the EU Science Hub https://joint-research-centre.ec.europa.eu/system/files/2022-07/finrep_fad-2021-0053_nutrixtend_mannanase.pdf
[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.

the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 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) and EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (EFSA, 2021).

3. Assessment

The present assessment regards the authorisation of the product consisting of β-mannanase (Enzyme Commission Number (EC) 3.2.1.78; 1,4-β-mannan mannanohydrolase) produced by *Aspergillus niger* CBS120604 as a zootechnical feed additive (functional group: digestibility enhancers) for all poultry for fattening. The product will be hereinafter referred to as Nutrixtend Optim, its trade name.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The β-mannanase is produced by a non-genetically modified *Aspergillus niger* strain, which is deposited at the Westerdijk Fungal Biodiversity Institute culture collection with deposit number CBS 120604.[6] The production strain has already been characterised in previous assessments (EFSA FEEDAP Panel, 2011, 2014).

The genome of the production strain was sequenced and used for identification purposes. The taxonomic identification of CBS 120604 as *A. niger* was confirmed by phylogenetic analysis using orthologous genes extracted from the BUSCO[7] database. The analysis included genomes of the genus *Aspergillus* section Nigri and grouped the production strain with the reference strain *A. niger* CBS 513.88.[8] The identification was further confirmed by ITS analysis and analysis of partial sequences of the calmodulin (*CaM*), β-tubulin (*benA*) and RNA polymerase II (*rpb2*) genes.[9]

The applicant investigated the capacity of the production strain to produce antimicrobials in four batches of the supernatant resulting from the fermentation.[10] 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 pyogenes* 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 were 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.

3.1.2. Manufacturing process

The active substance, β-mannanase, is produced by means of submerged fermentation by the non-genetically modified strain of *Aspergillus niger* CBS 120604.[12] [redacted] The applicant stated that no antimicrobial compounds are used during the manufacturing of the additive.

[6] Technical dossier/Section II/Annexes II_34 and 35.
[7] Benchmarking Universal Single-Copy Orthologs.
[8] Technical dossier/SIn_151222/Annexes II_80v2 and Annex I.
[9] Technical dossier/Section II/Annexes II_36 and 37.
[10] Technical dossier/Section II/Annexes II_12 and 13.
[11] Technical dossier/SIn_151222/Annex II_80v2.
[12] Technical dossier/SIn_151222/Annex II_39v2.

3.1.3. Characterisation of the additive

Nutrixtend Optim is an enzyme preparation with β-mannanase with a minimum activity of 265 U[13]/g. The formulation of the product includes [redacted] β-mannanase fermentation product and wheat flour [redacted] This product also contains inherent endo-1,(3)4-β-glucanase (EC 3.2.1.6) and endo-1,4-β-glucanase (EC 3.2.1.4) activity.[14]

Analytical data to confirm the specifications were provided for five batches of the additive, showing an average value of 283 (272–291) U/g. The total organic solids (TOS) content of these batches ranged from [redacted] w/w.[15]

Three of those batches were analysed for chemical impurities and the content of mycotoxins.[16] The contents of cadmium, lead, mercury and arsenic were below the limit of quantification (LOQ),[17] except for two batches with a content of cadmium ≤ 0.03 mg/kg. The applicant also provided data (one batch) on the content of tin (< 1 mg/kg), copper (1 mg/kg), melamine (< 0.50 mg/kg), cyanuric acid (< 1 mg/kg) and methylmercury (< 0.01 mg/kg).

In three batches of the final product[19] and one batch of the supernatant of the fermentation biomass,[18] the level of mycotoxins, including aflatoxins (B1, G1, B2, G2), ochratoxin A, zearalenone, fumonisins (B1 and B2) and deoxynivalenol, was below the LOQ.[20]

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (Co-planar PCBs) were analysed only in one batch of the final additive,[18] showing values below the corresponding LOQs. The calculated (upper bound) levels of dioxins and the sum of dioxins and dioxin-like-PCBs were 0.562 ng WHO-PCDD/F-TEQ/kg and 0.542 ng WHO-PCDD/F-PCB-TEQ/kg.

The specifications of the additive include values for total viable counts ([redacted]), total coliforms ([redacted]), *Salmonella* spp. ([redacted]), *E. coli* ([redacted]) and yeasts and moulds ([redacted]). The analysis of five batches of the additive confirmed the compliance with the specifications set for microbiological contamination, showing data on total viable counts (< 10 CFU/g), total coliforms (< 10 CFU/g), yeasts and moulds (average value of 10 (10–30) CFU/g), *E. coli* (not detected in 25 g) and *Salmonella* spp. (not detected in 25 g).[21] No data on the presence of *Enterobacteriaceae* were provided.

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 strain was investigated in three independent fermentation batches of the intermediate enzyme concentrate ([redacted]), each analysed in triplicate.[22] The product (100 mL) was filtered through a 0.45-μm filter impregnated with a non-selective medium and incubated at 30°C for 5 days. A positive control was included in the analysis. No viable cells of the production strain were detected in the samples tested.

3.1.4. Physical properties of the additive

The additive has a bulk density (average of three batches) of 655 kg/m^3.[23] The dusting potential of three batches of the additive was determined using the Stauber-Heubach method II and showed values on average of 670 mg/m^3 (range 660–679 mg/m^3) (mg airborne dust per m^3 of air).[24] The particle size of the dust was analysed by the laser-diffraction method in three batches of the additive;[25] the results showed that, on average (v/v), 0.66, 4.61 and 38.5% of the particles have a particle size < 10 μm, < 50 μm and < 100 μm, respectively.

[13] One unit of activity is defined as the amount of enzyme required to release one micromole of mannose reducing sugar equivalents per minute at 40°C at pH = 4.5.
[14] Average endo-1,4-β-glucanase activity of 5 batches: 776.7 U/g.
[15] Technical dossier/SIn_100323/Annexes II_1a to 5a.
[16] Technical dossier/Section II/Annexes II_20 to 22.
[17] LOQ (mg/kg): lead (0.05); mercury (0.005); cadmium (0.01); arsenic (0.04).
[18] Technical dossier/Section II/Annex II_20.
[19] Technical dossier/SIn_121022/Annexes II_21, 22 and 80.
[20] LOQ (μg/kg): aflatoxins (1.5); ochratoxin A (1); zearalenone (5); fumonisin B1 (5) and B2 (5); deoxynivalenol (100).
[21] Technical dossier/Section II/Annexes II_1 to 5.
[22] Technical dossier/Section II/Annex II_29.
[23] Technical dossier/Section II/Annexes II_77, 78 and 79.
[24] Technical dossier/Section II/Annex II_72.
[25] Technical dossier/Section II/Annexes II_30, 31 and 32.

3.1.5. Stability and homogeneity

3.1.5.1. Shelf-life

The applicant proposes a maximum shelf-life of 12 months at 18°C when kept in its original unopened container. The shelf-life of the additive (three batches) was studied when stored at 4 or 18°C in high-density polyethylene containers for 12 months. Losses at the end of the storage period were below 10%.[26]

3.1.5.2. Stability

The stability of the additive (three batches) in a vitamin–mineral premixture was studied when supplemented at 2% and stored at 4, 18 or 30°C in high-density polyethylene containers for 6 months. Losses at the end of the storage period were below 10%.[27]

The stability of the additive (three batches) in chickens for fattening fed mash feed was studied when supplemented to obtain enzyme activity of 27 or 53 U/kg feed, and stored at 4, 18 or 30°C in high-density polyethylene containers for 3 months. Losses at the end of the storage period were below 10%.

The stability of the additive (three batches) in chickens for fattening fed pelleted feed was studied when supplemented to obtain enzyme activity of 27 or 53 U/kg feed, and stored at 4, 18 or 30°C in high-density polyethylene containers for 3 months. Losses at the end of the storage period were below 10%.[28]

The effect of pelleting in the feed (two batches) was studied when pelleted at 70°C for 2 s or at 85°C for 25 s. Results showed losses after pelleting of 15.8 or 16.7%, respectively.[29]

3.1.5.3. Homogeneity

The homogeneous distribution of the additive in premixtures was studied in 10 subsamples when added at a 2% inclusion rate ([redacted]); the coefficient of variation was 7.0%. The homogeneous distribution of the additive in mash and pellets was studied in 10 subsamples of chickens for fattening feed (27 U/kg feed); the coefficient of variation was 10.1 (mash) and 10.7% (pellets).[30]

3.1.6. Conditions of use

The additive is intended for use in feed for all poultry for fattening at a proposed minimum use level of 27 and a maximum of 53 U/kg complete feed. The applicant recommends conditioning and pelleting temperatures not higher than 85°C.

3.2. Safety

3.2.1. Toxicological studies

In a previous opinion regarding the assessment of the additive [redacted],[31] the FEEDAP Panel evaluated one bacterial reverse mutation test, one *in vitro* chromosome aberration test, one *in vivo* mammalian erythrocyte micronucleus test and a 90-day oral toxicity test in rats for which the test item used was a solid intermediate enzyme product of *A. niger* CBS120604 (EFSA FEEDAP Panel, 2011). This solid intermediate enzyme product is the same one used to formulate [redacted] and Nutrixtend Optim. The applicant declared that the manufacturing process is the same and has not changed since the previous application. Data were provided to support that the test item used in the toxicological studies represents the solid intermediate enzyme product used to formulate Nutrixtend Optim: [redacted][32]
The Panel considers that the test item is representative of the final form of the additive under assessment. Thus, the conclusions reached in the previous opinion may also apply to the current assessment.

[26] Technical dossier/Section II/Annex II_48 and SIn_121022/Annex II_85.
[27] Technical dossier/Section II/Annex II_52 and SIn_121022/Annex II_85.
[28] Technical dossier/Section II/Annex II_52.
[29] Technical dossier/Section II/Annexes II_56 and 57.
[30] Technical dossier/Section II/Annex II_59.
[31] An authorised additive containing alpha-galactosidase and endo-1,4-beta-glucanase (4a17).
[32] Technical dossier/Section III/Annex III_16.

The bacterial reverse mutation test and the *in vitro* chromosome aberration tests assessed in the previous opinion showed that the test item did not induce gene mutations in bacteria and structural aberrations in human lymphocytes under the experimental conditions applied in each study, respectively (EFSA FEEDAP Panel, 2011). In the *in vivo* micronucleus test, no clinical signs of toxicity were reported, and the ratio of polychromatic/normochromatic erythrocytes evidenced no bone marrow toxicity. The frequency of micronuclei was comparable between the treated and control groups. Due to the lack of evidence of target tissue exposure, the FEEDAP Panel cannot extrapolate the negative results observed in the bone marrow to other organs (i.e., first sites of contact as stomach and liver) where concentrations higher than those reaching the bone marrow are expected. Therefore, the potential clastogenic and aneugenic effects of the test item at the first contact sites could not be addressed.

In the current application, an *in vitro* micronucleus test was submitted to evaluate the potential of the additive to induce structural and numerical chromosome aberrations.[33] The test item used was a liquid intermediate enzyme concentrate obtained from a different point of the manufacturing process than the one used for the other toxicological studies described above. Still, the test item showed higher β-mannanase activity than Nutrixtend Optim ([redacted]) and was considered representative of the final form of the additive. The *in vitro* micronucleus test was performed in Chinese Hamster Ovary cells (CHO-K1) according to OECD TG 487 and following good laboratory practices (GLP). 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 a β-mannanase activity of 11.15, 22.26 and 44.53 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. Cells were treated with 25, 50 and 100 μg TOS/mL, corresponding to an activity of β-mannanase ranging from 0.89 to 3.56 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 concluded that the test item did not induce structural and numerical chromosome aberrations under the experimental conditions applied in this study.

The subchronic oral toxicity study assessed in the previous opinion showed no relevant changes in haematology and clinical chemistry parameters nor treatment-related effects on organ weights or after gross and histopathology investigation (EFSA FEEDAP Panel, 2011). The no observed adverse effect level (NOAEL) derived from that study was 1,000 mg/kg body weight (BW) per day, the highest dose tested, corresponding to 23,420 U/kg BW per day.

3.2.1.1. Conclusions of the toxicological studies

The FEEDAP Panel concludes that the intermediate products used for the formulation of the additive showed no genotoxicity potential in tests addressing gene mutations, and numerical and structural chromosome aberrations. Moreover, from the results obtained in a subchronic oral toxicity study, a NOAEL of 23,420 U/kg BW per day was derived, the highest dose tested.

3.2.2. Safety for the target species

The applicant provided one tolerance trial in chickens for fattening and one in turkeys for fattening to support the safety of the additive for the target animals. However, the trial in turkeys for fattening was not considered further evidence of the safety of the target species due to the high mortality reported (average of 7%).[34]

3.2.2.1. Safety for chickens for fattening

A total of 480 1-day-old male chickens for fattening (Ross 308) were distributed in 60 pens and randomly allocated to five dietary treatments (12 replicates per group).[35] Three basal diets (starter, from day 1 to 9; grower, from day 10 to 20; and finisher from day 21 to 42) based on maize, soyabean meal and wheat were either not supplemented (control) or supplemented with Nutrixtend Optim to provide 28 (ca. 0.5× maximum recommended level), 55 (ca. 1×), 111 (ca. 2×) and 11,136

[33] Technical dossier/SIn_121022/Annex III_50.
[34] Technical dossier/Section III/Annex III_7.
[35] Technical dossier/Section III/Annex III_1.

(ca. 200×) U β-mannanase/kg feed. The analytical values of the enzymatic activity at levels of the maximum recommended one and above were slightly lower than the intended ones.[36] The experimental diets were offered ad libitum in pelleted form for 42 days. The grower diet included an external marker for digestibility analysis. Animals' health and mortality were checked daily, and the most probable cause of death/culling was recorded. The birds were weighed at the start of the trial, and, thereof, body weight and feed intake were recorded on days 11, 22 and 42. The total feed intake, body weight gain and feed-to-gain ratio were calculated and corrected for mortality for the starter, grower, finisher and the overall study period. On day 21, excreta samples were collected for 3 h from each pen. Feed and excreta samples were analysed for dry matter, nitrogen, gross energy and the external marker, and the apparent metabolisable energy corrected for nitrogen (AMEn) of the diet was calculated. The data were analysed with one-way analysis of variance (ANOVA), including the treatment as fixed effect. Group means were compared with Duncan's test. The significance level applied was 0.05.

Mortality including culling was 5.2, 4.2, 4.2, 4.2 and 3.1% for the control, 0.5×, 1×, 2× and 200× diets, respectively, and no difference was observed between groups. The supplementation of the chickens' diet with Nutrixtend Optim from 28 U/kg improved the feed-to-gain ratio in comparison with the control diet (control = 1.50; 0.5 × =1.45; 1 × =1.44; 2 × =1.42; 200 × =1.46). No differences between groups were detected in the final body weight (average of 3,007 g) and cumulative feed intake (4,322 g/bird) at the end of the trial.

The additive was tolerated at ~ 200 times the maximum recommended level. Therefore, the FEEDAP Panel concludes that the additive is safe at 53 β-mannanase U/kg complete feed for chickens for fattening, with a wide margin of safety. This conclusion can be extrapolated to all poultry for fattening.

3.2.2.2. Toxicological data

To support the safety of the additive for the target species, the applicant referred to the 90-day toxicity study above-mentioned (see Section 3.2.1). The NOAEL identified (23,430 β-mannanase U/kg BW per day) was used to calculate the maximum safe level for chickens and turkeys for fattening in accordance with the procedure described in the Guidance on the safety for the target species (EFSA FEEDAP Panel, 2017b). The values obtained for chickens (2,610 U/kg complete feed) and turkeys for fattening (3,515 U/kg complete feed) are two orders of magnitude higher than the recommended use level of 53 U/kg complete feed. Therefore, the Panel concludes that the additive is safe for all poultry for fattening.

3.2.2.3. Conclusions on Safety for the target species

Based on the data available, the FEEDAP Panel concludes that the additive is safe for all poultry for fattening at 53 U β-mannanase/kg complete feed.

3.2.3. Safety for the consumer

The results of toxicological studies (genotoxicity and subchronic oral toxicity studies) evaluated in the current and previous (EFSA FEEDAP Panel, 2011) opinions do not indicate any reason for concern for consumer safety arising from the use of the intermediate products used for the formulation of the additive.

3.2.4. Safety for the user

3.2.4.1. Effect on respiratory system

No specific studies were provided by the applicant regarding the effects of the additive on the respiratory system. Considering the proteinaceous nature of the active substance, the additive is considered a respiratory sensitiser.

3.2.4.2. Effect on eyes and skin

The potential for skin[37] and eye[38] irritation of the intermediate solid enzyme concentrate used to formulate the additive was tested in valid studies performed according to OECD guidelines 404 and

[36] Enzyme activity level (U β-mannanase/kg feed) in starter/grower/finisher: < LOQ/< LOQ/< LOQ, 19/24/31, 35/35/54, 72/101/87, and 9,036/8,239/9,698 for control, 0.5×, 1×, 2× and 200× groups, respectively.
[37] Technical dossier/Section III/Annex III_18.
[38] Technical dossier/Section III/Annex III_21.

405, respectively, showing that the test item is irritant to skin and eye. The results of a local lymph node assay performed with the intermediate solid enzyme concentrate following the OECD guideline 429 showed that the test item is a skin sensitiser under the conditions of the test.

3.2.4.3. Conclusions on safety for the user

Based on the studies submitted, the additive was shown to be an irritant to skin and eyes and to be a dermal sensitiser. Due to the proteinaceous nature of the active substance (β-mannanase), it is considered a respiratory sensitiser.

3.2.5. Safety for the environment

The active substance of the additive is a protein, and as such will be degraded/inactivated during passage through the digestive tract of animals or in the environment. Therefore, no risks to the environment are expected, and no further environmental risk assessment is required.

3.3. Efficacy

3.3.1. Efficacy for chickens for fattening

The applicant submitted one short-term trial and two long-term trials (including the tolerance efficacy trial described in Section 3.2.2.1) in chickens for fattening to support the efficacy of the additive in the target species.

In the short-term efficacy trial 1,[39] a total of 80 one-day-old male chickens for fattening (Ross 308) were distributed in pairs to 40 cages and randomly allocated to four treatments (10 replicates per treatment). The basal diet based on maize, wheat and soyabean meal was either not supplemented (control) or supplemented with Nutrixtend Optim to provide 30, 42 or 57 U β-mannanase/kg complete feed. The enzymatic activity of the experimental feeds was analytically confirmed.[40] From day 1 to 7 of age, birds were fed with the same basal diet. From day 8 to 21, the experimental diets were offered ad libitum in mash form. From day 17 to 21, excreta were collected by the total collection method and pooled per cage. Feed and excreta samples were analysed for the content of dry matter, nitrogen and gross energy and the AMEn was calculated. The body weight and feed intake of the birds were recorded at the start and end of the balance trial, and the feed-to-gain ratio was calculated. The experimental data were analysed with ANOVA, including the diet and block (location in the house) as fixed effects, and using the cage as the experimental unit. Mean groups were compared by Duncan's test. The significance level applied was 0.05. No effect of the diet on the performance of the birds was observed. The supplementation of the diet of chickens for fattening with Nutrixtend Optim at 30 and 57 U/kg feed showed higher AMEn than the control diet (11.9, 12.4, 12.1 and 12.5 for the control, 30, 42 or 57 U/kg groups, respectively).

The tolerance efficacy trial 2 (described above in Section 3.2.2.1) and the additional long-term efficacy trial 3 followed a similar design aiming at assessing the effects of the additive on the zootechnical parameters of the chickens for fattening during the whole productive cycle (42 days) and on the dietary AMEn during the grower phase.

In the efficacy trial 3,[41] 384 1-day-old male chickens for fattening (Ross 308) were distributed into 48 pens each containing eight birds, and randomly allocated to four treatments (12 replications/ treatment). The basal diets (starter, from day 1 to 11; grower, from day 11 to 22; and finisher, from day 22 to 42) based on maize, wheat and soyabean meal, were either not supplemented (control) or supplemented with Nutrixtend Optim to provide 30 or 57 U β-mannanase/kg complete feed. The enzymatic activity of the feeds was analytically confirmed.[42] A positive control with standard energy content according to recommendations for chickens for fattening was also considered. The experimental diets were offered ad libitum in the pelleted form up to day 42 of life and included an external marker for digestibility analysis.

Mortality and health status were monitored daily, and the most likely cause of death/culling was recorded. The birds were individually weighed at the start of the experiment (day 1). Thereafter, body weight and feed intake were recorded at every diet change (days 11 and 22) and at the end of the

[39] Technical dossier/SIn_121022/Annex IV_7.v2.
[40] β-mannanase activity: < LOQ, 23, 33 and 39 U/kg feed for the control, 30, 42 or 57 groups, respectively.
[41] Technical dossier/SIn_121022/Annex IV_13.v2.
[42] Starter/grower/finisher diets (U β-mannanase/kg feed): < LOQ/< LOQ/< LOQ, 21/26/32 and 46/35/48 for the control, 30 and 57 U/kg groups, respectively.

experiment (day 42). The total feed intake, body weight gain and feed-to-gain ratio were calculated and corrected for mortality for the starter, grower, finisher and overall study period. On day 21, excreta samples were collected for 3 h. Feed and excreta samples were analysed for the content of dry matter, external marker, nitrogen and gross energy, and the AMEn were calculated. Data were analysed by ANOVA, including the treatment as a fixed effect. Group means were compared with Duncan's test. Significance was set at 0.05.

The Panel notes that the excreta collection time during the balance trial both in the tolerance-efficacy trial 2 and in the long-term trial 3 (3 h) was not in line with the minimum period established in the Guidance for the assessment of the efficacy of feed additives (2018a). Therefore, these data were not considered for the assessment of efficacy. The results of the zootechnical performance of the birds of the two trials are shown in Table 1.

Table 1: Effects of the dietary supplementation with Nutrixtend Optim on the zootechnical performance of chickens for fattening

Trial	Groups	Total feed intake	Final body weight	Total weight gain	Feed-to-gain ratio	Mortality and culling
	(U/kg feed)	(g/bird)	(g)	(g)		(%)
2	0	4,412	2,974	2,936^{b}	1.50^{a}	5.20
	28	4,298	2,992	2,954^{ab}	1.46^{b}	4.17
	55	4,319	3,032	2,994^{a}	1.44^{b}	4.17
	111	4,240	3,015	2,977^{ab}	1.42^{b}	4.17
	11,136	4,354	3,024	2,986^{ab}	1.46^{ab}	3.13
3	0	4,778	3,138^{b}	3,097^{b}	1.55^{a}	5.21
	30	4,741	3,340^{a}	3,299^{a}	1.44^{b}	5.21
	57	4,703	3,328^{a}	3,287^{a}	1.46^{b}	4.17
	PC	4,649	3,375^{a}	3,332^{a}	1.39^{b}	3.13

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

In trials 2 and 3, the mortality and culling rates were in line with standard commercial production levels within the EU, with no differences between groups. The supplementation of the diet of chickens for fattening with Nutrixtend Option at the recommended use levels (from 27 to 53 U/kg feed) showed a better feed-to-gain ratio compared to the control group in both trials. In trial 3, higher final body weight and total weight gain were also observed at the same levels, while only in the group fed 55 U/kg feed in trial 2.

3.3.2. Conclusions on efficacy

The additive has the potential to be efficacious in chickens for fattening when added to feed at 30 U β-mannanase/kg complete feed. This conclusion can be extrapolated to all poultry for fattening.

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[43] and good manufacturing practice.

4. Conclusions

Nutrixtend Optim is safe for all poultry for fattening at the maximum recommended level of 53 U β-mannanase/kg feed.

The use of Nutrixtend Optim in animal nutrition is of no concern for consumer safety.

The use of the additive as a feed additive for all poultry for fattening is considered safe for the environment.

The additive is considered an irritant to skin and eyes and a dermal sensitiser. Due to the proteinaceous nature of the active substance (β-mannanase), it is considered a respiratory sensitiser.

[43] 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.

The Panel concludes that the additive has the potential to be efficacious in all poultry for fattening when added to feed at 30 U β-mannanase/kg complete feed.

References

EFSA (European Food Safety Authority), 2021. EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. EFSA Journal 2019;19(7):6506, 14 pp. https://doi.org/10.2903/j.efsa.2021.6506

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Scientific Opinion on the safety and efficacy of Biogalactosidase BL (alpha-galactosidase and beta-glucanase) as feed additive for chickens for fattening. EFSA Journal 2011;9(12):2451, 19 pp. https://doi.org/10.2903/j.efsa.2011.2451

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific opinion on the safety and efficacy of AGal-Pro (alpha-galactosidase and endo-1,4-beta-glucanase) as a feed additive for chickens reared for laying and minor poultry species for fattening. EFSA Journal 2013;11(7):3286, 8 pp. https://doi.org/10.2903/j.efsa.2013.3286

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014. Scientific Opinion on the safety and efficacy of AGal-Pro BL-L (alpha-galactosidase and endo-1,4-beta-glucanase) as a feed additive for chickens for fattening. EFSA Journal 2014;12(11):3897, 11 pp. https://doi.org/10.2903/j.efsa.2014.3897

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific Opinion on the safety and efficacy of AGal-Pro BL/B-L (alpha-galactosidase and endo-1,4-beta-glucanase) as a feed additive for laying hens and minor poultry species for laying. EFSA Journal 2015;13(5):4107, 11 pp. https://doi.org/10.2903/j.efsa.2015.4107

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Dujardin B, Galobart J and Innocenti ML, 2017a. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pp. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML, 2017b. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 12 pp. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017c. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2018a. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018b. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Bastos M, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brock T, de Knecht J, Kolar B, van Beelen P, Padovani L, Tarres-Call J, Vettori MV and Azimonti G, 2019. Guidance on the assessment of the safety of feed additives for the environment. EFSA Journal 2019;17(4):5648, 78 pp. https://doi.org/10.2903/j.efsa.2019.5648

Abbreviations

AMEn	Nitrogen-corrected apparent metabolisable energy
ANOVA	Analysis of Variance
BenA	β-tubulin
BUSCO	Benchmarking Universal Single-Copy Orthologs
BW	Body weight
CHO-K1	Chinese Hamster Ovary cells
PCBs	Polychlorinated biphenyls
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GLP	Good laboratory practices
LOQ	Limit of quantification
OECD	Organisation for Economic Co-operation and Development
PCDDs	Polychlorinated dibenzodioxins
PCDFs	Polychlorinated dibenzofurans
RPB2	RNA polymerase II
TOS	Total organic solids
WGS	Whole genome sequence