SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of endo-1,4-β-D-mannanase produced by *Thermothelomyces thermophilus* DSM 33149 (Natupulse® TS/TS L) for chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds (BASF SE)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of endo-1,4-β-D-mannanase produced by *Thermothelomyces thermophilus* DSM 33149 (Natupulse® TS/TS L) as a zootechnical feed additive for chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds. The additive under assessment, Natupulse® TS/TS L, does not pose any safety concern regarding the production strain. The FEEDAP Panel concluded that the additive is tolerated by chickens for fattening, and this conclusion can be extrapolated to all poultry for fattening. Due to the lack of reliable data regarding the potential of the additive to induce chromosomal damage, the FEEDAP Panel cannot conclude on the safety of the additive for the target species and on the safety for consumer. The use of the additive in animal nutrition is considered safe for the environment. The additive is considered not to be irritant to the skin and eyes but is considered a respiratory sensitiser, although exposure by inhalation is unlikely. The Panel could not conclude on the potential of the additive to be a skin sensitiser. Due to the lack of reliable data, the FEEDAP Panel considered that a potential of the additive to induce chromosomal damage in exposed unprotected users cannot be excluded. Consequently, exposure of users should be minimised. The Panel concluded that the additive Natupulse® TS/TS L has the potential to be efficacious in chickens for fattening at the proposed conditions of use and this conclusion can be extrapolated to turkeys for fattening, minor poultry species for fattening and ornamental birds.

Keywords: zootechnical additives, digestibility enhancers, endo-1,4-β-D-mannanase, Natupulse®, poultry, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2021-00075

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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 BASF SE[2] for the authorisation of the additive consisting of endo-1,4-β-D-mannanase produced by *Thermothelomyces thermophilus* DSM 33149 (Natupulse® TS/TS L), when used as a feed additive for chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds (category: zootechnical additives; 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 1 March 2021.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the endo-1,4-β-D-mannanase produced by *T. thermophilus* DSM 33149 (Natupulse® TS/TS L), when used under the proposed conditions of use (see **Section 3.1.5**).

1.2. Additional information

The subject of the assessment is the product consisting of endo-1,4-β-D-mannanase produced by *T. thermophilus* DSM 33149, intended for use as a zootechnical additive (functional group: digestibility enhancers) for chickens for fattening, turkeys for fattening, minor poultry species for fattening and ornamental birds. The additive has not been authorised in the European Union.

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[3] in support of the authorisation request for the use of endo-1,4-β-D-mannanase produced by *T. thermophilus* DSM 33149 (Natupulse® TS/TS L) as a feed additive. The dossier was received on 11/12/2020 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00075.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 5/3/2021 to 3/6/2021 for which received comments that were considered for the assessment.

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-D-mannanase in animal feed.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of endo-1,4-β-D-mannanase produced by *T. thermophilus* DSM 33149 (Natupulse® TS/TS L) is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance

[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] BASF SE, Carl-Bosch Straße 38, 67,056 Ludwigshafen, Germany.
[3] FEED dossier reference: FAD-2020-0107.
[4] Evaluation report received on 27/05/2021 and available on the EU Science Hub: https://joint-research-centre.ec.europa.eu/publications/fad-2020-0107_en
[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.

on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP 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) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The additive under assessment consists of endo-1,4-β-D-mannanase (IUBMB EC 3.2.1.78; mannanase), produced by a genetically modified strain of *T. thermophilus* (DSM 33149) and is intended to be used as a zootechnical additive (functional group: digestibility enhancer) for chickens for fattening, turkeys for fattening, minor poultry species for fattening and ornamental birds. It will be hereafter referred to as Natupulse® TS/TS L, its trade name.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The endo-1,4-β-D-mannanase is produced by a genetically modified strain of *T. thermophilus* which is deposited [redacted].[6]

The identity of the production strain as *T. thermophilus* was confirmed [redacted].[7]

The whole genome sequence (WGS) data of the production strain was queried against a total of 91 fungal sequences coding for toxins identified in the UniProt Toxin-Database. Results using tBLASTn, showed only two hits but with low homologies (< 42% identity) with the killer toxin subunit alpha (subunit not toxic in itself) and cerato-platanin (a phytotoxin with both toxic activity and ability to elicit plant protective responses).[8] No hits of concern were identified.

3.1.1.1. Information related to the genetically modified microorganism

Characterisation of the recipient microorganism

[redacted]

Description of the genetic modification

[redacted]

[6] Technical dossier/Section II/Annex II_117.
[7] Technical dossier/Section II/Annex II_122.
[8] Technical dossier/Section II/Annex II.122.

[redacted]

The absence of any antimicrobial resistance gene [redacted].[9,10]

3.1.2. Manufacturing process

The active substance of the additive is produced by [redacted][11] [redacted][12] [redacted] The obtained concentrate (hereafter referred to Mannanase concentrate) is used to formulate the solid and liquid forms of the additive.[13]

The solid formulation (Natupulse® TS) is obtained by mixing the Mannanase concentrate [redacted][14] [redacted].[15]

The liquid formulation (Natupulse® TS L) is obtained by mixing the Mannanase concentrate [redacted].[7]

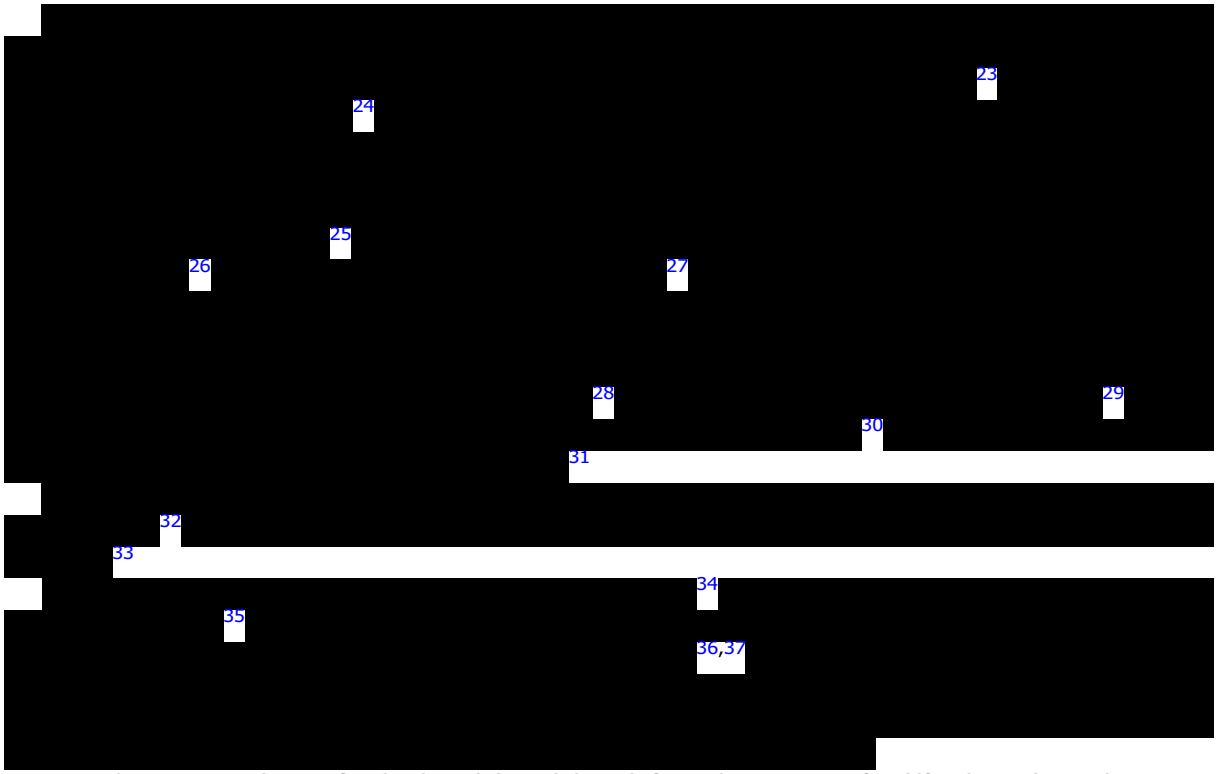
3.1.3. Characterisation of the additive

The additive is available in two formulations with a minimum enzymatic activity of 8,000 Thermostable Mannanase Unit (TMU)[16]/g:

- Natupulse® TS (solid formulation): [redacted].[17]
- Natupulse® TS L (liquid formulation): [redacted].[18]

The applicant provided analysis [redacted][19] [redacted][20] [redacted][21] [redacted].[22]

[9] Technical dossier/Supplementary Information December 2022/Annex_SIn_II_7.
[10] Technical dossier/Supplementary Information December 2022/Annex_SIn_II_5 and Annex_SIn_II_6.
[11] Technical dossier/Section II/Annex II_137.
[12] Technical dossier/Section II/Annex II_138.
[13] Technical dossier/Supplementary information December 2021/Suppl_Info_Natupulse and Annex_SIn_2.
[14] Technical dossier/Supplementary information December 2021/Annex_SIn_4.
[15] Technical dossier/Supplementary information December 2021/Suppl_Info_Natupulse.
[16] One Thermostable Mannanase Unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one micromole mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 ± 0.1°C and pH 3.5.
[17] Technical dossier/Section II/Annex II_12.
[18] Technical dossier/Section II/Annex II_16.
[19] Technical dossier/Section II/Annex II 20, Annex II 21, Annex II 23 and Annex II 24 and Supplementary Information December 2021/Annex_SIn_19 and Annex_SIn_21.
[20] Technical dossier/Section II/ Annex II 26, Annex II 27, Annex II 28, Annex II 29 and Annex II 30.
[21] Technical dossier/Section II/Annex II 20, Annex II 21, Annex II 23 and Annex II 24 and Supplementary Information December 2021/Annex_SIn_19 and Supplementary Information December 2021/Annex_SIn_21.
[22] Technical dossier/Section II/Annex II_1 and Annex II_2.



Formulation ingredients for both solid and liquid formulations are food/feed grade and are not expected to contribute with undesirable impurities.

The detected amounts of the above described impurities do not raise safety concerns.

The presence of the production organism was investigated in triplicate in five pilot batches[38] and three production batches of the Mannanase concentrate.[39]

[redacted]

[23] Technical dossier/Section II/Annex II_36, Annex II_37, Annex II_38, Annex II_39 and Annex II_40.
[24] Technical dossier/Section II/Annex II_41, Annex II_42, Annex II_43, Annex II_44 and Annex II_45.
[25] Technical dossier/Section II/Annex II_51, Annex II_52, Annex II_53, Annex II_54 and Annex II_55.
[26] Limit of detection (LOD): aflatoxin B1 0.3 μg/kg; Ochratoxin A 5 μg/kg; T-2 toxin 0.5 μg/kg; zearalenone 1 μg/kg; sterigmatocystin 10 μg/kg. HT-2 toxin 0.2 ppm; fumonisins B1 and B2 20 μg/kg; deoxynivalenol 10 μg/kg.
[27] Limit of quantification: arsenic 0.04 mg/kg, cadmium 0.01 mg/kg, fluorine 10 mg/kg, lead 0.1 mg/kg and mercury 0.001 mg/kg.
[28] Technical dossier/Section II/Annex II_58, Annex II_59, Annex II_60, Annex II_61 and Annex II_62.
[29] Technical dossier/Section II/Annex II_63, Annex II_64, Annex II_65, Annex II_66 and Annex II_67.
[30] Technical dossier/Section II/Annex II_68, Annex II_69, Annex II_70, Annex II_71 and Annex II_72.
[31] Technical dossier/Section II/Annex II_73, Annex II_74, Annex II_75, Annex II_76 and Annex II_77.
[32] Parameters tested: Lead (<5 mg/kg), cadmium (<0.5 mg/kg), arsenic (<2 mg/kg), mercury (<0.1 mg/kg), fluorine (<150 mg/kg), aflatoxin B1 (<0.5 μg/kg), zearalenone (<1 μg/kg), ochratoxin A (<5 μg/kg), T2 toxin (<0.5 μg/kg), sterigmatocystin (<10 μg/kg), *Salmonella* spp. (no detection in 25 ml), *Escherichia coli* (no detection in 25 ml), coliform bacteria (no detection), total microbial count (<50 CFU/ml) and yeasts and filamentous fungi (<50 CFU/ml).
[33] Technical dossier/Section II/Annex II_78, AnnexII_79 and Annex II_80.
[34] Technical dossier/Section II/Annex_II_83, Annex_II_84, Annex_II_85, Annex_II_86, Annex_II_87, Annex_II_88, Annex_II_89, Annex_II_93, Annex_II_94 and Annex_II_95.
[35] Technical dossier/Section II/Annex_II_96, Annex_II_97, Annex_II_98, Annex_II_99, Annex_II_100, Annex_II_101, Annex_II_102, Annex_II_103, Annex_II_107, Annex_II_108, Annex_II_109, Annex_II_110 and Annex_II_111.
[36] Parameters tested for the solid formulation: lead (<1 mg/kg), aflatoxin B1 (<0.3 μg/kg), deoxynivalenol (283–311 μg/kg), zearalenone (1–2 μg/kg), HT2 toxin (<5 μg/kg), T2 toxin (<1 μg/kg), fumonisins B1 and B2 (<20 μg/kg), ochratoxin A (<5 μg/kg), *Salmonella* spp. (not detected in 25 g), *Escherichia coli* (not detected in 25 g), coliform bacteria (<0.3 CFU/g), total microbial count (0.25–3.25 × 10^3 CFU/g).
[37] Parameters tested for the liquid formulation: lead (<0.1 mg/kg), cadmium (<0.01 mg/kg), arsenic (<0.04 mg/kg), mercury (<0.001 mg/kg), aflatoxin B1 (<0.3 mg/kg), deoxynivalenol, zearalenone (<1 μg/kg), HT2 toxin (<5 μg/kg), T2 toxin (<1 μg/kg), ochratoxin A (<5 μg/kg), fumonisins B1 and B2 (<20 μg/kg), sterigmatocystin (tested in only three batches; <10 μg/kg), *Salmonella* spp. (not detected in 25 ml), *Escherichia coli* (not detected in 25 ml), coliform bacteria (<1 CFU/ml), total microbial count (<50 CFU/ml) and yeasts and filamentous fungi (<50 CFU/ml).
[38] Technical dossier/Section II/Annex II_56.
[39] Technical dossier/Section II/Annex II_81.

[redacted] No viable cells of the production strain were detected.

The presence of DNA of the production strain was tested [redacted][40][41][42][43][44] No amplified DNA was obtained in any of the batches tested.

No viable cells or DNA of the production strain were found in the intermediate product, and since this is more concentrated than the additive, the conclusions on these results apply also to the additive.

[redacted][7][45][46][47][48] No inhibitory activity was found.

3.1.3.1. Physical properties of the additive

[redacted][49][50][51][52]

3.1.4. Stability and homogeneity

The shelf life of the additive was tested with the liquid formulation and with a spray-dried intermediary product (corresponding to the Mannanase concentrate with magnesium sulfate followed by spray-drying) instead of the solid formulation Natupulse® TS.

The stability of the additive in premixture was studied with the spray-dried intermediary product only. The stability of the additive in feedingstuffs, and its capacity for homogenous distribution in feed were studied with both the solid and liquid formulations.

The materials added to the spray-dried product to prepare the solid formulation (basically wheat bran/middlings), are not expected to modify the stability properties of the mannanase, therefore such data can be used to study the stability of Natupulse® TS.

[redacted]

[40] Technical dossier/Section II/Annex II_57.
[41] Technical dossier/Section II/Annex II_82 and Supplementary Information December 2022/ Annex_SIn_II_3 and Annex_SIn_II_4.
[42] Technical dossier/Supplementary Information December 2022/Annex_SIn_II_1 and Annex_SIn_II_2.
[43] Technical dossier/Spontaneous Information/Annex_SInAd_2.
[44] Technical dossier/Supplementary Information December 2022/Annex_SIn_II_2.
[45] Technical dossier/Section II/Annex_II_46, Annex_II_47, Annex_II_48, Annex_II_49, Annex_II_50, Annex II_78, AnnexII_79 and Annex II_80.
[46] Technical dossier/Section II/Annex_II_90, Annex_II_91 and Annex_II_92.
[47] Technical dossier/Section II/Annex II_104, Annex_II_105 and Annex_II_106.
[48] Technical dossier/Section II/Annex II_130.
[49] Technical dossier/Section II/Annex II_115.
[50] Technical dossier/Section II/Annex II_113.
[51] Technical dossier/Supplementary Information December 2021/Annex_SIn_45.
[52] Technical dossier/Section II/Annex II_116.

3.1.5. Conditions of use

The additive is intended for use in feed for chickens for fattening, turkeys for fattening, minor poultry species for fattening and ornamental birds at a proposed use level of 800 TMU/kg complete feed.

3.2. Safety

3.2.1. Safety of the production organism

The production strain *T. thermophilus* DSM 33149 was developed from *T. thermophilus* VKM F-3500-D and expresses a synthetic codon-optimised mannanse gene from *T. reesei*. The introduced sequences raise no safety concern and there are no antibiotic resistance genes remaining from the genetic modification process. Based on the data submitted, the strain is not expected to produce any

[53] Technical dossier/Section II/Annex II_153 and Supplementary Information December 2021/Annex_SIn_12, Annex_SIn_20 and Annex_SIn_22.
[54] Technical dossier/Section II/Annex II_154 and Supplementary Information December 2021/Annex_SIn_49.
[55] Technical dossier/Section II/Annex II_155 and Supplementary Information December 2021/Annex_SIn_50.
[56] Technical dossier/Section II/Annex II_156.
[57] Technical dossier/Section II/Annex II_157.
[58] Technical dossier/Section II/Annex II_158.
[59] Technical dossier/Section II/Annex II_160.
[60] Technical dossier/Section II/Annex II_161.

toxic compound or have antimicrobial activity. The production strain and its DNA were not detected in an intermediate product representative of both final formulations of the additive. The additive formulations Natupulse® TS/TS L, manufactured with the production strain *T. thermophilus* DSM 33149, do not give rise to safety concerns regarding the genetically modified production strain.

3.2.2. Toxicological studies

3.2.2.1. Genotoxicity studies, including mutagenicity

3.2.2.1.1. Bacterial reverse mutation test

[redacted][61] The Panel concluded that the Mannanase concentrate did not induce gene mutations in bacteria under the experimental conditions applied in the study.

3.2.2.1.2. In vitro *mammalian cell micronucleus test*

[redacted][62] No cytotoxicity and no increase in the frequency of micronuclei were observed in binucleated cells after treatment with the test item in any experimental condition.

Based on the available data and the deviation observed from OECD TG 487, the Panel considered the study not reliable to evaluate the potential of the test item to induce chromosomal damage.

3.2.2.2. Subchronic toxicity study (90-day study)

[redacted][63][64] No-treatment-related adverse effects were observed in animals at any level of the additive.

[61] Technical dossier/Section III/Annex_III_6 and Supplementary Information December 2021/Annex_SIn_32.
[62] Technical dossier/Section III/Annex_III_7 and Supplementary Information December 2021/Annex_SIn_32.
[63] Technical dossier/Section III/Annex III_8.
[64] Technical dossier/Supplementary Information December 2021/Annex SIn_52.

Therefore, the no observed adverse effect level (NOAEL) for Natupulse® TS L is determined to be 15,000 mg TOS /kg, the highest concentration tested, or a mean daily intake of 1,117 mg/kg bw per day in male and 1,298 mg/kg bw per day in female rats.

3.2.2.3. Conclusions on the toxicological studies

The FEEDAP Panel concluded that there is no concern regarding gene mutation induction by the fermentation product. However, the Panel cannot conclude on its potential to induce chromosomal aberrations due to the lack of reliable data.

The FEEDAP Panel identified from the sub-chronic oral toxicity study a NOAEL for Natupulse® TS L of 15,000 mg TOS /kg, the highest concentration tested.

3.2.3. Safety for the target species

3.2.3.1. Safety for chickens for fattening

[redacted][65]

3.2.3.2. Conclusions on safety for the target species

Based on the results obtained in a tolerance trial, the FEEDAP Panel concludes that Natupulse® TS is tolerated by chickens for fattening at the recommended use level of 800 TMU/kg complete feed. Considering the wide margin of safety (125×) shown in the tolerance trial in chickens for fattening, the FEEDAP Panel can extrapolate the conclusion for turkeys for fattening, for minor poultry species for fattening and for ornamental birds for growing at the same recommended level. However, the FEEDAP Panel cannot conclude on the potential of the additive to induce chromosomal aberrations and therefore the Panel cannot conclude on the safety for the target species.

3.2.4. Safety for the consumer

The FEEDAP Panel considered that the subchronic oral toxicity study did not show any adverse effects (see Section 3.2.2.2) and that there is no concern regarding gene mutation induction of the fermentation product (see Section 3.2.2.1.1).

However, the Panel cannot conclude on the potential to induce chromosomal aberrations due to the lack of reliable data, and therefore, the FEEDAP Panel could not conclude on the safety for consumer.

[65] Technical dossier/Section III/Annex III_3_IRTA_2019.

3.2.5. Safety for the user

3.2.5.1. Effect on respiratory system

[redacted][66] However, the FEEDAP Panel notes that the study was performed with an intermediate of the solid formulation of the additive, and not with the final formulation as requested by the relevant Guidance.

Owing to the nature of the active substance, the additive should be considered a respiratory sensitiser; however, considering the absence of dust in the solid formulation, the likelihood of exposure is low.

3.2.5.2. Effect on eyes and skin

[redacted][67] [redacted][68] Results showed no skin irritation potential under the test conditions (UN GHS Classification"No Category").

[redacted][69] [redacted][70] [redacted][71] However, this standalone study cannot be considered to conclude on the potential for skin sensitisation. According to the OECD Guideline 497, the results from this assay should be complemented by other *in chemico* or *in vitro* methods and combined with *in silico* information to provide an unequivocal conclusion.

[redacted][72] [redacted][73] [redacted] The test item did not show an eye irritation potential under the test conditions chosen (UN GHS Classification "No Category").

3.2.5.3. Conclusions on safety for the user

The additive is not irritant to the eyes or skin. The FEEDAP Panel cannot conclude on the potential of the additive to be a skin sensitiser. Owing to the proteinaceous nature of the active substance, the additive should be considered a respiratory sensitiser.

Due to the lack of reliable data, the FEEDAP Panel could not conclude on the potential genotoxicity of the additive. Therefore, the exposure to the additive of the unprotected users should be minimised.

3.2.6. Safety for the environment

The production strain and its DNA were not detected in the intermediate concentrate that is representative of the final formulations. The final product does not pose any environmental safety concern associated with the genetic modification. The active substance of the additive is a protein and as such it will be degraded/inactivated during the passage through the digestive tract of the animals or in the environment. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

[66] Technical dossier/Section III/Annex III_16.
[67] Technical dossier/Supplementary Information December 2021/Annex SIn 57.
[68] Technical dossier/Supplementary Information December 2021/Annex SIn 59.
[69] Technical dossier/Supplementary Information December 2021/Annex SIn 60.
[70] Technical dossier/Supplementary Information December 2021/ Annex SIn 61.
[71] Technical dossier/Supplementary Information December 2021/Annex SIn 60 and Annex SIn 61.
[72] Technical dossier/Supplementary Information December 2021/Annex SIn 54.
[73] Technical dossier/Supplementary Information December 2021/Annex SIn 58.

3.3. Efficacy

3.3.1. Efficacy for chickens for fattening

One short-term trial (trial 1)[74] and two long-term trials (trials 2 and 3)[75] were provided for the assessment.

Short-term trial

[redacted]

Long-term trials

[redacted]

Table 1: Trial design and use level of the long-term efficacy trials performed in chickens for fattening

Trial	Total n° of animals (animals × replicate) Replicates × treatment	Breed Sex (duration)	Composition feed (form)	Groups (TMU/kg feed)	
				Intended	Analysed
[77]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[78]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

[74] Technical dossier/Supplementary Information December 2021/Annex_SIn_62.
[75] Technical dossier/Section IV/Annex IV_3 and Annex IV_4.
[76] Technical dossier/Supplementary Information December 2021/Annex SIn_65.
[77] Technical dossier/Section IV/Annex IV_3.
[78] Technical dossier/Section IV/Annex IV_4.

Table 2: Effects of Natupulse® TS on the performance of chickens for fattening

Trial	Groups (TMU/kg feed)	Daily feed intake (g)	Final body weight (g)	Daily body weight gain (g)	Feed to gain ratio (g/g)	Mortality and culling (%)
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

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

3.3.1.1. Conclusions on efficacy

A positive effect was observed in the balance study with an improvement in the apparent metabolisable energy content in the diets offered to the chickens for fattening. In the two long-term studies, a positive effect was observed regarding the daily body weight gain and the final body weight of the chickens for fattening and in one of the trials on the feed to gain ratio.[79]

The Panel concludes that the additive has the potential to be efficacious for chickens for fattening when added to feed at 800 TMU/kg feed. The conclusion reached in chickens for fattening can be extrapolated to turkeys for fattening, minor poultry species for fattening and ornamental birds at a proposed use level of 800 TMU/kg feed.

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[80] and Good Manufacturing Practice.

4. Conclusions

The production strain is a genetically modified strain of *T. thermophilus* (DSM 33149). No viable cells nor DNA of the production strain were detected in an intermediate concentrate that is representative of the final formulations. Therefore, the additive under assessment, Natupulse® TS/TS L, does not pose any safety concern regarding the production strain.

The FEEDAP Panel cannot conclude on the safety of the additive for the target species and the consumer, due to the lack of reliable data for the assessment of the potential genotoxicity of the additive.

The use of the additive in animal nutrition is safe for the environment.

The additive is considered to be not irritant to the eyes and skin, but a respiratory sensitiser, although exposure by inhalation is unlikely. The FEEDAP Panel cannot conclude on the potential of the additive to be a skin sensitiser. Due to the lack of reliable data, the FEEDAP Panel could not conclude on the potential of the additive to induce chromosomal aberrations. Therefore, the exposure to the additive of the unprotected users should be minimised.

The additive is considered to be efficacious in feedingstuffs for chickens for fattening, turkeys for fattening, minor poultry species for fattening and ornamental birds at a use level of 800 TMU/kg.

References

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

[79] Technical dossier/Section IV/ Annex IV_4.

[80] 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.

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

BW	body weight
CFU	colony-forming unit
CV	coefficient of variation
DM	dry matter
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
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
RH	relative humidity
WGS	Whole Genome Sequence