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SCIENTIFIC OPINION

Safety 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, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety of a feed additive consisting of endo-1,4-β-D-mannanase produced by *Thermothelomyces 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 safety and efficacy of the additive have been already assessed previously; however, the FEEDAP Panel could not conclude on the safety of the additive for the target species, consumers and the users due to lack of reliable data on the potential genotoxicity of the additive. In the present assessment, the applicant submitted a new *in vitro* mammalian cell micronucleus test. After the assessment of the data newly submitted, the FEEDAP Panel concluded that the use of the feed additive in animal nutrition under the conditions of use proposed is of no concern for target species and consumer safety. The additive is not irritant to the eyes or skin. Owing to the proteinaceous nature of the active substance, the additive should be considered a respiratory sensitiser. The Panel cannot conclude on the potential of the additive to be a skin sensitiser.

KEYWORDS

digestibility enhancers, endo-1,4-β-D-mannanase, genotoxicity, safety, user, Zootechnical additives

CONTENTS

1 | INTRODUCTION

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

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition and article 9 defines the terms of the authorisation by the Commission.

The Applicant, BASF SE, is seeking a Community authorisation 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 (Table 1).

TABLE 1 Description of the additive.

Category of additive	
Functional group of additive	Zootechnical additive
Description	Endo-1,4-β-D-mannanase produced by *Thermothelomyces thermophilus* DSM 33149 (Natupulse® TS/TS L)
Target animal category	Chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds
Applicant	BASF SE
Type of request	New opinion

On 01.02.2023, The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of the European Food Safety Authority (EFSA), in its opinion on the safety and efficacy of 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 came to an inconclusive opinion.

The Commission gave the possibility to the applicant to submit supplementary information and data to complete the assessment and allow a revision of the EFSA's opinion. The new data have been received by the Commission on 26.07.2023 on the e-submission food chain platform (application number FEED-2023-17,170).

In view of the above, the Commission asks EFSA to deliver a new opinion on Natupulse® TS/TS L as a zootechnical feed additive for chickens and turkeys for fattening, minor poultry species for fattening and ornamental birds based on the supplementary information and data submitted by the applicant, in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002.

1.2 | Additional information

The product consisting of endo-1,4-β-D-mannanase produced by *Thermothelomyces 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, is not currently authorised in the European Union.

In 2023, the FEEDAP Panel adopted an opinion on the safety and efficacy of the additive under assessment (EFSA FEEDAP Panel, 2023).

2 | DATA AND METHODOLOGIES

2.1 | Data

The present assessment is based on data submitted by the applicant in the form of supplementary information[2] to a previous application on the same product.[3] The dossier was received on 9/8/2023 and the general information and supporting documentation are available on Open.EFSA at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00545.

In accordance with Article 38 of the Regulation (EC) No 178/2002[4] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[5] a non-confidential version of the supplementary information has been published on Open.EFSA.

[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]Dossier reference: EFSA-Q-2023-00545.

[3]Dossier reference: FAD-2020-0107.

[4]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[5]Decision available online: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

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' knowledge, to deliver the present output.

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 *Thermothelomyces thermophilus* DSM 33149 is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017) and Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017).

3 | ASSESSMENT

The additive Natupulse® TS/TS L 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 at a proposed use level of 800 Thermostable Mannanase Units (TMU)[7]/kg complete feed. It will be hereafter referred to as Natupulse® TS/TS L.

The additive is available in two formulations, one solid (Natupulse® TS) and one liquid (Natupulse® TS L), which ensure a minimum enzymatic activity of 8000 TMU/g.

In the previous assessment (EFSA FEEDAP Panel, 2023), the Panel concluded regarding the target species that the additive Natupulse® TS is tolerated by chickens for fattening at the recommended use level of 800 TMU/kg complete feed with a wide margin of safety (125x) and this conclusion was extrapolated to turkeys for fattening, for minor poultry species for fattening and for ornamental birds at the same recommended level.

Regarding the safety for the consumers, the FEEDAP Panel concluded that the subchronic oral toxicity study did not show any adverse effects (No Observed Adverse Effect Level-NOAEL was 15,000 mg TOS/kg, the highest concentration tested) and that there was no concern regarding gene mutation induction of the fermentation product. However, the Panel considered the *in vitro* mammalian cell micronucleus test submitted by the applicant, as not reliable to evaluate the potential of the test item to induce chromosomal damage.

Regarding the safety for the users, the FEEDAP Panel concluded that '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'.

Considering the lack of reliable data on genotoxicity, the FEEDAP Panel could not conclude on the safety for the target species, the consumers and the users.

In the current dossier, the applicant submitted additional data to address the gaps identified in the previous assessment regarding the genotoxicity potential of the additive.

3.1 | Safety

For the previous assessment (EFSA FEEDAP Panel, 2023), the potential of a mannanase concentrate used to formulate the additive ([redacted]) to induce gene mutations and chromosomal damage was studied in a bacterial reverse mutation test conducted in compliance with the OECD Testing Guideline (TG) 471 and in an *in vitro* mammalian cell micronucleus test. The FEEDAP Panel concluded that there is no concern regarding gene mutation induction by the fermentation product. However, considering the data available and the deviation from OECD TG 487, the Panel considered the *in vitro* mammalian cell micronucleus test as not reliable to evaluate the potential of the test item to induce chromosomal damage.

For the current assessment, the applicant provided a new *in vitro* mammalian cell micronucleus test,[8] which is described below.

The ability of the mannanase concentrate ([redacted]) to induce chromosomal damage was evaluated in an *in vitro* micronucleus assay performed in human peripheral blood lymphocytes in line with OECD TG 487

[6]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.

[7]One Thermostable Mannanase Unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to 1 μM mannose from locust bean gum (0.3 g/100 mL buffer solution) in 1 min under the assay conditions of 50.0 0.1°C and pH 3.5.

[8]Annex_ComplInfo_4_[redacted]_2023_conf.pdf.

and claimed to be good laboratory practice (GLP) compliant. Cells were exposed to 653, 1143 or 2000 µg/mL active ingredient/endo-1,4-β-D-mannanase applying a short treatment (3 + 25 h of recovery) in the presence and absence of metabolic activation, and a continuous treatment (28 h) without metabolic activation. Cytochalasin-B (CytB) was added during the recovery period in the short treatment, while co-treatment of CytB and test item was carried out in the continuous treatment. No induction of cytotoxicity was observed in the treated samples with respect to the concurrent vehicle controls. Comparable frequencies of micronuclei were observed between treated and vehicle control cultures.

The FEEDAP Panel concluded that the test item does not raise concern for the induction of structural and numerical chromosomal aberrations.

For the current assessment, the applicant has not provided any additional data in support of the safety for the users other than the genotoxicity test.

3.1.1 | Conclusions on safety

The newly submitted genotoxicity test shows that the additive does not raise concerns regarding its genotoxicity potential.

Considering the above and taking into account the tolerance and toxicological studies already assessed in the previous opinion (EFSA FEEDAP Panel, 2023), the FEEDAP Panel concludes that the additive Natupulse® TS/TS L is safe for chickens for fattening, turkeys for fattening, minor poultry species for fattening and ornamental birds, and for the consumer.

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

4 | CONCLUSIONS

The FEEDAP Panel concludes that Natupulse® TS/TS L is safe for chickens for fattening, turkeys for fattening, minor poultry species for fattening and ornamental birds at the proposed use level.

The use of the additive as a feed additive raises no concerns for the consumers.

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

ABBREVIATIONS

CytB	Cytochalasin-B
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practice
OECD	Organisation for Economic Co-operation and Development
TMU	Thermostable Mannanase Unit

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2023-00545

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