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Safety and efficacy of Natugrain® TS/TS L (endo-1,4-beta-xylanase and endo-1,4-beta-glucanase) as a feed additive for sows

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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 and efficacy of Natugrain® TS/TS L. The additive is a preparation of endo-1,4-beta-xylanase and endo-1,4-beta-glucanase produced by two genetically modified strains of Aspergillus niger, and it is authorised for use in piglets (weaned) and pigs for fattening, poultry species and ornamental birds. The applicant requested the extension of use of the additive to sows at 560 TXU and 250 TGU/kg feed. The FEEDAP Panel concluded that there are no concerns for consumer safety and no risks for the environment are expected from the use of the additive in sows. The additive should be considered a potential respiratory sensitiser for the users. Considering the results from a subchronic oral toxicity study and the tolerance study in lactating sows, the Panel concluded that the additive is safe for sows at the recommended dose. The data submitted to support the efficacy for sows allowed the Panel to conclude that the additive is efficacious in lactating sows, data on gestating sows were not given, and therefore, the Panel could not conclude on the efficacy of the additive in that physiological stage.

Keywords: Zootechnical additive, digestibility enhancers, xylanase, glucanase, safety, efficacy sows

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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 a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from BASF SE[2] for authorisation of the product Natugrain® TS/TS L (endo-1,4-beta-xylanase and endo-1,4-beta-glucanase), when used as a feed additive for sows (category: zootechnical additive; functional group: digestibility enhancers). The application was made for "sows in order to have benefits in piglets but the applicant requested during the assessment to limit it to sows including gestating and lactating.

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). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 20 October 2017.

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 product Natugrain® TS/TS L (endo-1,4-beta-xylanase and endo-1,4-beta-glucanase), when used under the proposed conditions of use (see Section 3.1).

1.2. Additional information

The additive Natugrain® TS/TS L is a preparation of endo-1,4-beta-xylanase and endo-1,4-beta-glucanase produced by two genetically modified strains of *Aspergillus niger* (CBS 109.713 and DSM 18404). The European Food Safety Authority (EFSA) issued an opinion on the safety and efficacy of this enzyme preparation when used in piglets (weaned), chickens for fattening, laying hens, turkeys for fattening and ducks for fattening, including the assessment of the safety for the consumer, the user and the environment, as well as the safety aspects of the genetic modification (EFSA, 2008). The FEEDAP Panel adopted four further opinions on the use of this product as a zootechnical additive in other avian species (EFSA FEEDAP Panel, 2011a), in pigs for fattening (EFSA FEEDAP Panel, 2013) in laying hens (EFSA FEEDAP Panel, 2014) and for chickens reared for laying and minor poultry species for laying purposes (EFSA FEEDAP Panel, 2016).

This product is authorised for use in piglets (weaned) and pigs for fattening, poultry species and ornamental birds.[3,4,5]

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[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

[2] BASF SE, ENS/LR – F31, 68623 Lampertheim, Germany.

[3] Commission Regulation (EC) No 271/2009 of 2 April 2009 concerning the authorisation of a preparation of endo-1,4-beta-xylanase and endo-1,4-beta-glucanase as a feed additive for weaned piglets, chickens for fattening, laying hens, turkeys for fattening and ducks for fattening (holder of the authorisation BASF SE). OJ L 91, 3.4.2009, p. 5. Amended by Commission implementing regulation (EU) No 1070/2014 of 10 October 2014 amending Regulation (EC) No 271/2009 as regards the minimum content of the preparation of endo-1,4-beta-xylanase by *Aspergillus niger* (CBS 109.713) and endo-1,4-beta-glucanase produced by *Aspergillus niger* (DSM 18404) as a feed additive for laying hens holder of authorisation BASF SE). OJ L 295, 11.10.2014, p. 49.

[4] Commission Implementing Regulation (EU) No 1068/2011 of 21 October 2011 concerning the authorisation of an enzyme preparation of endo-1,4-beta-xylanase produced by *Aspergillus niger* (CBS 109.713) and endo-1,4-beta-glucanase produced by *Aspergillus niger* (DSM 18404) as a feed additive for chickens reared for laying, turkeys for breeding purposes, turkeys reared for breeding, other minor avian species (other than ducks for fattening) and ornamental birds (holder of authorisation BASF SE). OJ L 277, 22.10.2011, p. 11. Amended by Commission implementing regulation (EU) 2017/950 of 2 June 2017 amending Implementing Regulation (EU) No 1068/2011 as regards the minimum content of the preparation of endo-1,4-beta-xylanase produced by *Aspergillus niger* (CBS 109.713) and endo-1,4-beta-glucanase produced by *Aspergillus niger* (DSM 18404 as a feed additive for chickens reared for laying and all avian species for laying (holder of authorisation BASF SE). OJ L 143, 3.6.2017, p. 5.

[5] Commission Implementing Regulation (EU) No 1404/2013 of 20 December 2013 concerning the authorisation of a preparation of endo-1,4-beta xylanase produced by *Aspergillus niger* (CBS 109.713) and endo-1,4-beta-glucanase produced by *Aspergillus niger* (DSM 18404) as a feed additive for pigs for fattening (holder of authorisation BASF SE). OJ L 349, 21.12.2013, p. 88.

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 Natugrain® TS/TS L (endo-1,4-beta-xylanase and endo-1,4-beta-glucanase) 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.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the active substances in animal feed 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 Natugrain® TS/TS L is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011a,b), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

Natugrain® TS/TS L is a feed additive that contains endo-1,4-beta-xylanase (EC 3.2.1.8; xylanase) and endo-1,4-beta-glucanase (EC 3.2.1.4; glucanase) and is intended to be used in sows as a zootechnical additive (functional group: digestibility enhancers).

3.1. Characterisation

The additive Natugrain® TS is available in solid (TS) and liquid formulations (TS L) and contains xylanase and glucanase. The enzymes are produced separately by two genetically modified strains of *Aspergillus niger*. The production strain of the xylanase is deposited at the Centraalbureau voor Schimmelcultures with the accession number CBS 109.713 and the production strain of the glucanase is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen with the deposit number DSM 18404.[9] [redacted] The product has been characterised in detail in a previous opinion (EFSA, 2008) and no new data have been provided. Therefore, the data pertaining to composition, physical properties and stability submitted in the previous assessment still apply.

The additive is to be used in feed for sows at recommended activities of 560 TXU and 250 TGU per kg feed.[12]

3.2. Safety

Safety aspects regarding the use of this additive in feed including the safety of the genetic modification of the production strain, the safety for the consumers, for the users and for the environment have been previously assessed (EFSA, 2008). The Panel concluded that there are no concerns for consumer safety and no risks for the environment are expected. The Panel also concluded

[6] FEED dossier reference: FAD-2017-0050.
[7] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2010-0034.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] Technical dossier/Section II/Annex II.115.
[10] One TXU is defined as the amount of enzyme that liberates 5 μmol of reducing sugars (xylose equivalents) from wheat arabinoxylan per minute at pH = 3.5 and 55°C.
[11] One TGU is defined as the amount of enzyme that liberates 1 μmol of reducing sugars (glucose equivalents) from barley betaglucan per minute at pH = 3.5 and 40°C.
[12] Technical dossier/Supplementary information July 2019.

that the additive in both forms is not irritant to eyes or skin, but it is potential skin and respiratory sensitiser.

The dossier does not contain any new information that would lead the FEEDAP Panel to reconsider the conclusions drawn previously. Moreover, the FEEDAP Panel considers that the new use in sows would not introduce any risk/hazard that has not been already considered. However, there is the need to assess the safety of the additive for the new target species.

3.2.1. Safety for the target species

The applicant provided a subchronic oral toxicity study and a tolerance trial in lactating sows in order to support the safety for the target species.

The subchronic oral toxicity study has been evaluated by the FEEDAP Panel previously (EFSA 2008) and the no observed adverse effect level (NOAEL) identified was 200,000 TXU and 90,000 TGU/kg body weight (bw) and day.[13] Using the NOAEL identified in the study, and applying the procedure detailed in the guidance on the safety for the target species (EFSA FEEDAP Panel, 2017), the maximum safe level in feed for sows is calculated to be approximately 58,000 TXU and 26,000 TGU/kg feed, corresponding to more than 100 times the recommended dose.

The applicant also provided a tolerance trial in lactating sows [redacted] The Panel noted some limitations in the reporting of the data/methods obtained/followed, including: missing information on the farrowing performance of the sows, no statistical analysis on the body weight of the sows, the litter size at the end of the study was not clearly indicated[redacted] and litter weight at the end of the study was not provided. Moreover, the study did not address other reproductive parameters since it was done only during the lactation phase. However, the results obtained did not show any adverse effect of the additive on the parameters monitored and reported when fed to sows at 100-fold the recommended dose.

Considering that the maximum safe level in feed for sows calculated from the subchronic oral toxicity study corresponded to more than 100 times the recommended dose, as well as the results from the study in lactating sows in which a 100-fold did not show adverse effects on the sows and litters during the lactation phase, the Panel concludes that the additive is safe for the target species at the recommended level.

3.3. Efficacy

The applicant submitted four efficacy studies which aimed mainly at determining the content of metabolisable energy of the diet (trials 1 and 2) or the apparent faecal digestibility of the energy (trials 3 and 4) in lactating sows, zootechnical performance parameters were also measured.

[redacted] and Supplementary information July 2019.

[redacted], but no clear information was provided.

The first study is the tolerance trial presented in Section 3.2.1. The second study[redacted] was done in the same trial site as the first study and followed the same study design and limitations indicated for the tolerance trial regarding the reporting of some information (i.e., sows' body weight, litter size and total litter weight). In total, 20 sows were included and were offered either a non-supplemented diet (control) or the control diet supplemented with Natugrain® TS to provide (xylanase/glucanase) 560/250 TXU/TGU per kg feed.

The third and fourth studies were conducted in the same trial site, and shared the same study design.[redacted]

The results of the four studies showed improvements on the metabolisable energy content of the diet (trials 1 and 2) or on the faecal apparent digestibility of the energy (trial 3) when sows were fed the additive at the recommended level compared to the control. No other significant effects were found between the recommended level and the control in any of the parameters measured, with the exception of a higher body weight of the piglets in trials 1 and 2. In trial 1, the piglets from sows receiving 56,000 TXU and 25,000 TGU/kg feed showed higher body weights compared to the control and use level group. In trial 2, the piglets in the control had a higher final body weight compared to the treated group. The Panel considers that the limitations identified in the reporting in trials 1 and 2 and the results on the growth of the piglets would not have an impact on the conclusions drawn regarding the metabolisability/digestibility of the energy of the diet.

Table 1: Effect of Natugrain® TS on the daily feed intake, body weight of the sows during the lactation and on the metabolisability/digestibility of the energy of the diet

Trial	Group (TXU-TGU/kg feed)	Daily feed intake (g)	Initial body weight (kg)(1)	Final body weight (kg)	Metabolisable Energy content of the diet (MJ/kg feed)	Faecal apparent digestibility of energy (%)
1	0-0	5,511	314	265	12.3[b]	–
	560-250	5,637	310	257	12.9[a]	–
	56000-25000	5,404	315	262	12.9[a]	–
2	0-0	6,800	277	245	12.7[b]	–
	560-250	6,500	279	240	13.1[a]	–
3	0-0	5,170	291	256	–	79.9[b]
	560-250	5,200	293	262	–	81.2[a]
4	0-0	3,830	270	227	–	82.9
	560-250	4,280	260	227	–	83.1

(1): Body weight in trials 1 and 2 is prior to farrowing, in trials 3 and 4 it is after farrowing.

[redacted]

Table 2: Effect of Natugrain® TS on litter size, piglets' weight and mortality

Trial	Group (FXU/kg feed)	Litter size (n)		Piglets' weight (kg)		Mortality/culls (%)(2)
		Initial(1)	Final	Initial(1)	Final	
1	0-0	13	–	1.95	7.02[b]	12.1
	560-250	13	–	1.96	7.09[b]	5.4
	56000-25000	13	–	1.89	7.48[a]	10.0
2	0-0	13	–	2.23	7.84[a]	12.3
	560-250	13	–	2.28	7.57[b]	3.8
3	0-0	12.4	10.8	1.66	8.73	11.8
	560-250	12.8	11.0	1.61	8.14	13.5
4	0-0	13.2	11.7	1.54	7.29	11.0
	560-250	12.7	11.1	1.50	7.16	11.8

(1): Initial litter size and piglets' weight on day 4 post-farrowing in trials 1 and 2 and day 1 in trial 3.
(2): Values for trials 1 and 2 are calculated from the raw data provided by the applicant.

Considering the results obtained in lactating sows, the FEEDAP Panel concludes that the additive has a potential to be efficacious as a zootechnical additive in improving the utilisation of the energy of the diets in sows at the lactating phase at the dose of 560 TXU and 250 TGU/kg feed. No data has been provided to allow the Panel to conclude on the efficacy during the gestation.

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[19] and Good Manufacturing Practice.

4. Conclusions

Natugrain® TS/TS L is safe for sows at 560 TXU and 250 TGU/kg feed.

The FEEDAP Panel concludes that there are no concerns for consumer safety and no risks for the environment are expected from the use of the additive in sows. The additive is not irritant to skin or eyes, but it is considered a potential skin and respiratory sensitiser.

The Panel concludes that the additive has the potential to be efficacious as a zootechnical additive for lactating sows at 560 TXU and 250 TGU/kg feed, however, cannot conclude on the efficacy for gestating sows due to the lack of data.

Documentation as provided to EFSA/Chronology

Date	Event
06/09/2017	Dossier received by EFSA. Natugrain® TS/TSL. Submitted by BASF SE
08/09/2017	Reception mandate from the European Commission
20/10/2017	Application validated by EFSA – Start of the scientific assessment
15/02/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety and efficacy for the target species*
03/06/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
06/09/2017	Comments received from Member States
29/01/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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

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Abbreviations

bw	body weight
EURL	European Union Reference Laboratory
FEEDAP	Additives and Products or Substances used in Animal Feed
NOAEL	No observed adverse effect level (NOAEL)