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SCIENTIFIC OPINION

Safety and efficacy of a feed additive consisting of endo-1,4-β xylanase, endo-1,4-β-glucanase and xyloglucan-specific-endo-β-1,4-glucanase produced by *Trichoderma citrinoviride* DSM 33578 (Huvezym® neXo) for all *Suidae* (Huvepharma EOOD)

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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 Huvezym® neXo 100 G/L, a product containing endo-1,4-β-xylanase, endo-1,4-β-glucanase and xyloglucan-specific-endo-β-1,4-glucanase activities produced by a non-genetically modified strain of *Trichoderma citrinoviride* (DSM 33578), as a zootechnical additive for all *Suidae*. The applicant provided information confirming the taxonomic identification of the production strain. The batches used for the characterisation of the final formulations showed compliance with the minimum specifications of the additive in terms of enzyme activities (> 15,000 EPU, > 1000 CU, > 1000 XGU per g) but not for the ratio of the enzymes, which is lower (ca. 7:1:1) than the ones specified (15:1:1). The Panel could not conclude on the representativeness of the test item used in the toxicological and tolerance studies with respect to the final formulations. Therefore, the conclusions are limited to the product with a minimum enzyme activity of 15,000 EPU, 1000 CU, 1000 XGU per g and a xylanase:glucanase:xyloglucanase ratio of 15:1:1. The Panel concluded that the additive is safe for the target species, consumers and the environment. Huvezym® neXo 100 G is not an irritant to the skin and eyes but should be considered a skin sensitiser. Huvezym® neXo 100 L is neither an irritant to the skin and eyes nor a skin sensitiser. Due to the proteinaceous nature of the active substances, the additive is considered a respiratory sensitiser. The additive has the potential to be efficacious in all reproductive *Suidae* at the minimum proposed use level. Owing to the lack of sufficient data, the Panel could not conclude on the efficacy of the additive for *Suidae* for fattening or reared for reproduction.

KEYWORDS

digestibility enhancers, efficacy, Huvezym® neXo 100 G/L, safety, *Trichoderma citrinoviride*, zootechnical additives

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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 Huvepharma EOOD[2] for the authorisation of the additive consisting of endo-1,4-β-xylanase, endo-1,4-β-glucanase and xyloglucan-specific-endo-β-1,4-glucanase produced by *Trichoderma citrinoviride* DSM 33578 (Huvezym® neXo), when used as a feed additive for all *Suidae* (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). 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 17 February 2023.

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 endo-1,4-β xylanase, endo-1,4-β-glucanase and xyloglucan-specific-endo-β-1,4-glucanase produced by *Trichoderma citrinoviride* DSM 33578 (Huvezym® neXo), when used under the proposed conditions of use (see **Section 3.1.3**).

1.2 | Additional information

The additive contains endo-1,4-β-xylanase, endo-1,4-β-glucanase and xyloglucan-specific-endo-β-1,4-glucanase produced by *Trichoderma citrinoviride* DSM 33578, and it is intended to be used in all *Suidae*.

EFSA issued one opinion on the safety and efficacy of this product when used in feed for poultry species, ornamental birds and piglets (weaned and suckling) (EFSA FEEDAP Panel, 2022).

The additive is currently authorised for use in the feed of poultry for fattening, poultry reared for laying/breeding, and ornamental birds.[3]

2 | DATA AND METHODOLOGIES

2.1 | Data

The present assessment is based on the data submitted by the applicant in the form of a technical dossier[4] in support of the authorisation request for the use of endo-1,4-β xylanase, endo-1,4-β-glucanase and xyloglucan-specific-endo-β-1,4-glucanase agent produced by *Trichoderma citrinoviride* DSM 33578 (Huvezym® neXo) as a feed additive. The dossier was received on 19 May 2022, and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00326.

In accordance with Article 38 of Regulation (EC) No 178/2002[7] and taking into account the protection of confidential information and 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,[8] a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements for the pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 6 December to 27 December 2023 for which no comments were received.

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.

[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]Huvepharma EOOD, 3A Nikolay Haytov str, 1113, Sofia (Bulgaria).
[3]COMMISSION IMPLEMENTING REGULATION (EU) 2023/1169 of 15 June 2023 concerning the authorisation of a preparation of endo-1,4-β-xylanase, endo-1,4-β-glucanase and xyloglucan-specific endo-β-1,4-glucanase produced by *Trichoderma citrinoviride* DSM 33578 as a feed additive for poultry for fattening, poultry reared for laying and reared for breeding, and ornamental birds (holder of authorisation: Huvepharma EOOD).
[4]Dossier reference: FEED-2022-3111.

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 substance/agent in animal feed/marker residue in tissues are valid and applicable to the current application.[5]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of endo-1,4-β-xylanase, endo-1,4-β-glucanase and xyloglucan-specific-endo-β-1,4-glucanase produced by *Trichoderma citrinoviride* DSM 33578 (Huvezym® neXo) 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, 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).

3 | ASSESSMENT

This opinion assesses the safety and efficacy of the product that contains endo-1,4-β-xylanase (xylanase, E.C.3.2.1.8), endo-1,4-β-glucanase (glucanase, E.C.3.2.1.4) and xyloglucanspecific-endo-β-1,4-glucanase (xyloglucanase, E.C.3.2.1.151) produced by *T. citrinoviride* DSM 33578 as a zootechnical additive (functional group: digestibility enhancers) for all *Suidae*. The additive under assessment will be hereafter referred to as Huvezym® neXo.

3.1 | Characterisation

The FEEDAP Panel has issued one opinion on the safety and efficacy of the same product when used in feed for poultry species, ornamental birds and piglets (weaned and suckling) (EFSA FEEDAP Panel, 2022). Most of the information in the characterisation section is considered to apply to the current assessment. The new information provided by the applicant is described below.

3.1.1 | Characterisation of the production microorganism

The enzymes present in the additive are produced by a non-genetically modified strain of *T. citrinoviride* which is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) with the accession number DSM 33578.[7] In the previous assessment, the applicant provided data to exclude the capacity of the production strain to produce antimicrobials, the presence of secondary metabolites and the presence of viable cells of the production organism (EFSA FEEDAP Panel, 2022). However, the taxonomic identification was not fully achieved because the sequence parameters used were considered not appropriate for *Trichoderma* identification.

In the current application, the applicant provided a new taxonomic identification analysis to address this limitation.[8] The whole genome of the production strain was sequenced and used for taxonomic identification purposes. The taxonomic identification of DSM 33578 as *T. citrinoviride* was confirmed by phylogenetic analyses using the marker genes Internal Transcribed Spacers (ITS) 1 and 2, the translation elongation factor 1 alfa (*tef1*) and the ribonucleic acid (RNA) polymerase subunit II (*rpb2*) gene sequences. The analysis showed that the production strain clusters together with the type material *T. citrinoviride* CBS 258.85.

3.1.2 | Characterisation of the additive

The additive is available in two different formulations: granulated (Huvezym® neXo 100 G) and liquid (Huvezym® neXo 100 L). Both formulations have a guaranteed minimum activity per gram of product of 15,000 xylanase units (EPU), 1000 glucanase units (CU) and 1000 xyloglucanase units (XGU) (enzyme ratio = 15:1:1). The manufacturing process and data

[5]Evaluation report available on the EU Science Hub: https://joint-research-centre.ec.europa.eu/publications/fad-2021-0036_en

[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]Annex_II_15_strain deposit letter.

[8]Annex_102_Phylogenetic analysis strain rpb2 ITS etf1 markers.

regarding the characterisation of the additive, including the batch-to-batch variation, microbial contamination, chemical impurities, physico-chemical properties, stability and homogeneity, have been previously assessed by the FEEDAP Panel and are considered to apply to the present assessment (EFSA FEEDAP Panel, 2022).

In the previous opinion, the FEEDAP Panel noted that:

(i) the intermediate enzyme concentrate used to prepare the final formulations of the additive, as described in the manufacturing process, is specified to contain minimum enzyme activities of [redacted] EPU/CU/XGU (enzyme ratio of ca. [redacted]). The analytical data provided on three batches of the enzyme concentrate showed compliance with those specifications and[9]

(ii) the final formulations (Huvezym neXo 100 G and 100 L) described in the characterisation section showed average enzyme activities of 21,917/3320/3178 (100 G) and 20,258/3202/3152 (100 L) EPU/CU/XGU (enzyme ratio of ca. 7:1:1).[10] These results would be in line with the dilution of the intermediate enzyme concentrate as per the manufacturing process. However, the Panel noted that the enzyme ratios are different from those set in the specifications (15:1:1).

The Panel notes that considering that the same production strain produces all the enzyme activities in a single fermentation process, it would be expected that the ratio of the enzyme activities found in the final formulations would be similar to that of the intermediate concentrate but with lower enzyme activity, as a consequence of the dilution by the rest of the formulated ingredients.

3.1.3 | Conditions of use

The additive is intended for use in feed for all *Suidae* at a proposed minimum level of 1500 EPU, 100 CU and 100 XGU/kg complete feed.

3.2 | Safety

The Panel notes that the test item used in the toxicological and tolerance studies[11] consisted of an enzyme concentrate showing a xylanase:glucanase:xyloglucanase ratio of, approximately, 13–15:1:1. This ratio is not in compliance with that of the intermediate concentrate described in the manufacturing process (ca. [redacted]) and of the final formulations as described in the characterisation section (ca. 7:1:1).

The different analytical enzyme ratios observed between the final forms of the additive and the test item used for the toxicological and tolerance studies suggest that those are obtained from separate production processes, which may lead to a different composition of the fermentation product. Therefore, the Panel cannot conclude on the representativeness of the test item used in the toxicological and tolerance studies with respect to the final formulations.

In the current application, the applicant has provided no new information that would explain these differences. Therefore, the Panel considers that the conclusions of the Safety section below are limited to a product with a minimum enzyme activity of 15,000 EPU, 1000 CU and 1000 XGU per gram and with a xylanase:glucanase:xyloglucanase ratio of ca. 15:1:1, in agreement with the product described in the specifications. No conclusions can be established on the safety of the final formulations (Huvezym neXo 100 G and 100 L) analysed in the characterisation section.

3.2.1 | Safety for the target species

The applicant submitted one tolerance trial in weaned piglets and one in sows, and the calculation of the maximum safe levels in feed using the No Observed Adverse Effect Level (NOAEL) derived from a 90-day study in rats to support the safety for the target animals.

The tolerance trial in weaned piglets was already assessed by the FEEDAP Panel in a previous opinion (EFSA FEEDAP Panel, 2022). The FEEDAP Panel disregarded the trial due to the high rate of morbidity (> 27%) observed during the trial, suggesting a poor health status of the animals. No new data on weaned piglets have been submitted. In that same assessment, the NOAEL from a 90-day study in rats allowed the Panel to conclude on the safety of the additive for piglets (suckling and weaned) at the proposed minimum level of 1500 EPU, 100 CU and 100 XGU per kg complete feed (for piglets, see also Table 1).

[9]Annex_II_39_CoA active substance.

[10]Annex_II_05_COAs Huvezym neXo 100 G; Annex_II_06_COAs Huvezym neXo 100 L.

[11]RSI EFSA-Q-2021-00308 FAD-2021-0036 consumer safety; Enzymes activity per gram of product: [redacted] (in vitro mammalian cell micronucleus test and sub-chronic oral toxicity study) and of [redacted] (bacterial reverse mutation test)

3.2.1.1 | *Tolerance trial in sows*

A total of 79 sows (Norsvin Landrace × Large White; from first to sixth parity) were used in the trial from Day 1 of gestation until weaning[12]. Until day 108 of gestation, the sows were kept in groups and then were moved to the lactation barns and housed individually. The sows were randomly allocated into three groups based on the supplementation level of the additive: two basal diets (gestation, based on wheat, wheat middling and soybean hulls; lactation, based on wheat, wheat middling and sunflower meal) were either not supplemented (control; 26 sows) or supplemented with the test item to provide 1500/100/100 (1× minimum use level; 25 sows) or 300,000/20,000/20,000 (200×; 28 sows) EPU/CU/XGU per kg feed. All the enzyme activities were confirmed in the experimental feeds.[13] The experimental diets were offered in pellet form. During the gestation period, all sows received a restricted amount of feed adapted to the gestation nutritional requirements, corresponding to ca. 2.8 kg feed/day. After farrowing, feed administration was adjusted according to the days of lactation, the weight of the sow and the number of suckling piglets per sow. The piglets received a creep feed from day 14 of life to weaning.

The general health status of the animals was monitored daily throughout the experiment, including the medical treatments administered. Sows' body weight (BW) and backfat (BF) thickness were measured at the start of the experiment (Day 1), at transfer to the farrowing pen (day 108) and at the end of weaning (day 142 of trial); the variation on the backfat thickness between the different time points was calculated. Individual feed consumption was measured daily throughout the whole trial. The evaluation of the farrowing performance included number of piglets born (alive, stillborn and dead) and BW of the piglets. Cross-fostering of piglets was done among sows of the same group during the first 48 h of life to equalise the number of piglets between litters and BW within litter. The litter performance was assessed by recording piglets' BW at birth, after cross-fostering (when relevant) and at weaning, as well as the number of weaned piglets and the creep feed intake. The weaning-to-oestrus interval was recorded for each sow.

The data on sow's feed intake and total born piglets were subjected to a non-inferiority test. For that purpose, the lower limits of the 95% confidence interval of the 1× and 200× diets mean minus the control were compared with the established margins for each parameter (feed intake = 0.5 kg/day; total born piglets = 2). The experimental data were analysed with a generalised linear model, including the treatment as a fixed effect, and, depending on the parameter analysed, different covariates were considered.[14] When differences were observed, group means were compared with Tukey's test. The significance level was set at 0.05.

Nine sows were excluded from the trial during the gestation/farrowing period due to abortion (one from 1×), low feed intake (one from control), small litter size at birth (one from control and two from the 1× and 200× groups) and other reasons not specified (one from control and 200×). One sow from 1× group died during the lactation period, and two others were removed due to low feed intake (control) and litter genetic abnormality (1× group).

Based on the confidence intervals obtained for the overall feed intake (−0.41 and −0.44 for 1× and 200× groups, respectively) and total born piglets (−1.28 and − 1.36), none of the supplemented groups showed to be inferior to the control group. Regarding the sows' performance, no differences were observed between the supplemented groups and the control for the gestation, lactation or overall period (feed intake – control value for lactation = 4.8 kg/day, overall = 3.3 kg/day; BW gain – gestation = 58.0 kg, lactation = −54.6 kg, overall = 4.8 kg; and backfat thickness variation – gestation = 3.26 mm, lactation = −2.71 mm, overall = 0.88 mm). Likewise, no differences were observed due to the dietary supplementation with Huvezym neXo® at any level in the sows' farrowing performance (length of gestation = 115.6 days; total live born piglets = 13.6) and of the litter during lactation (litter live weight at birth = 20.8 kg; at weaning = 98.5 kg; weaning age = 26.0 days; piglet creep feed intake = 43.6 g/day; mortality rates = 3.5%; number of weaned piglets = 12.7). The weaning-to-oestrus interval did not differ between groups (4.8 days).

Based on the results of the tolerance trial in sows, the Panel concludes that Huvezym neXo® is safe for sows at 1500/100/100 EPU/CU/XGU per kg complete feed, with a wide margin of safety. This conclusion can be extrapolated to all reproductive *Suidae*.

3.2.1.2 | *Toxicological data*

The results of the sub-chronic oral toxicity study in rats assessed previously by the Panel were used to support the safety for the target species (EFSA FEEDAP Panel, 2022). The resulting NOAEL (1000 mg/kg bw per day representing 1,181,000 EPU, 88,900 CU and 82,300 XGU/kg bw per day) was used to calculate the maximum safe level in feed for the different categories of *Suidae* following the procedure described in the Guidance on the safety for the target species (EFSA FEEDAP Panel, 2017b). The calculated maximum safe concentrations in feed for the new categories are presented in Table 1.

[12]Annex_III_01_TAS sows

[13]Gestation diets: 346-228-174, 1946-360-314, and 336,000-24,200-23,820 EPU-CU-XGU/kg complete feed for the control, 1×, and 200× groups, respectively. Lactation diets: 430-143-113, 2137-283-267, and 227,000-23,000-23,567 EPU-CU-XGU/kg complete feed for the control, 1×, and 200× groups, respectively.

[14]For the analysis of BW and BF loss, the initial BW and BF; for litter weight at birth, the total piglets born; and for pre-weaning litter growth and mortality, the number of piglets at standardisation and weaning age.

TABLE 1 Maximum safe levels in feed derived from toxicological data.

	Body weight (kg)	Feed intake (kg DM/day)	Daily feed intake (g DM/kg bw)	Maximum safe level (EPU-CU-XGU/kg feed)[a]
Weaned piglets	20	0.88	44	236,200–17,680 – 16,460
Pigs for fattening	60	0.176	37	283,444–21,336–19,752
Sow lactating	175	0.106	30	344,458–25,929 – 24,004

Abbreviations: CU, glucanase units; EPU, xylanase units; XGU, xyloglucanase units.
[a]Complete feed containing 88% dry matter.

The maximum safe levels obtained are higher than the proposed minimum use level of 1500 EPU, 100 CU and 100 XGU/kg complete feed for weaned piglets, pigs for fattening and lactating sows.

3.2.1.3 | *Conclusions on safety for the target species*

The FEEDAP Panel concludes that the product containing a minimum of 15,000 EPU, 1000 CU and 1000 XGU per gram and with a xylanase:glucanase:xyloglucanase ratio of ca. 15:1:1 is safe for weaned piglets, pigs for fattening and sows at the proposed conditions of use. These conclusions are extrapolated to all *Suidae*.

3.2.2 | Safety for the consumer

The safety of the product for the consumer was evaluated in the previous assessment (EFSA FEEDAP Panel, 2022). The FEEDAP Panel concluded that the product containing a minimum of 15,000 EPU, 1000 CU and 1000 XGU per gram and a xylanase:glucanase:xyloglucanase ratio of ca. 15:1:1 is safe for the consumers. The proposed use of the additive to the new species/categories would not introduce risks not already evaluated in the previous opinion.

3.2.3 | Safety for the user

Based on the data provided in the previous application (EFSA FEEDAP Panel, 2022), the Panel concluded that Huvezym® neXo 100G is neither corrosive to the skin nor an eye irritant but should be considered a potential skin sensitiser, while Huvezym® neXo 100L is neither corrosive to the skin nor a skin sensitiser and or an eye irritant. Due to the lack of data, no conclusions could be drawn on the skin irritation of the final formulations of the additive. Due to the proteinaceous nature of the active substances, the additive is considered a respiratory sensitizer.

In the context of the current application, the applicant submitted two in vitro tests to evaluate the skin irritancy potential of the final formulations of the additive (Huvezym neXo 100 G[15] and 100 L[16]), according to the OECD Test Guideline 439. The batches of the final formulations used were compliant with the specifications both in terms of enzyme activities and ratio (ca. 15:1:1). The results of the tests indicated that the final formulations of the additive Huvezym neXo 100 G and 100 L are not irritant to skin.

The FEEDAP Panel concludes that the product containing minimum enzyme activities of 15,000 EPU, 1000 CU and 1000 XGU per gram and with a xylanase:glucanase:xyloglucanase ratio of 15:1:1 is safe for users.

3.2.4 | Safety for the environment

The safety of the product for the environment was evaluated in the previous assessment (EFSA FEEDAP Panel, 2022). The active substances of the additive are proteins, 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. The proposed use of the additive to the new species/categories would not introduce risks not already evaluated in the previous opinion.

3.3 | Efficacy

The applicant submitted three trials in weaned piglets and three in sows to support the efficacy for the target species.

[15]Add_III.1_Skin_irritation_neXo_100_G.
[16]Add_III.1_Skin_irritation_neXo_100_L.

3.3.1 | Efficacy for weaned piglets

The three trials in piglets were assessed in the previous assessment of the Panel (EFSA FEEDAP Panel, 2022). At that time, one of the trials was not considered in the assessment due to the high number of medical treatments applied during the experiment ([redacted]) and the high mortality and culling rate ([redacted]). The other two trials were considered valid and showed improved performance (better feed-to-gain ratio) of the piglets when receiving the additive at the proposed use level of 1500 EPU, 100 CU and 100 XGU/kg complete feed. In the absence of a third trial showing positive effects, the Panel could not conclude on the efficacy of the additive in weaned piglets. The applicant did not provide any new information to support the efficacy of the additive in weaned piglets.

3.3.2 | Efficacy for sows

The applicant submitted three trials to support the efficacy of the additive in gestating (trials 1 and 2) and lactating (trials 1, 2 and 3) sows. The design of the trials is summarised in Table 2, and the main results are in Tables 3 (sows' performance and digestibility during gestation), 4 (sows' performance and digestibility during lactation) and 5 (litter performance).

TABLE 2 Trial design and use level of the efficacy trials performed in sows.

Trial	Number of animals (per replicate) replicates × treatment	Phase	Composition feed (form)	Groups(EPU-CU-XGU/kg feed)	
				Intended	Analysed
1[17]	[redacted]	Gestation	[redacted]	0–0-0 1500–100–100	330-90-100 2380-220-220
		Lactation	[redacted]		330-130-90 1790-350-230
2[18]	[redacted]	Gestation	[redacted]	0-0-0 1500-100-100	250-70-90 1840-240-210
		Lactation	[redacted]		200-100-85 1570-295-230
3[19]	[redacted]	Lactation	[redacted]	0-0-0 1500-100-100	280-80-95 1420-220-215

Abbreviations: CU, glucanase units, EPU, xylanase units, XGU, xyloglucanase units.

In trial 1, [redacted][20] [redacted][21]

[17]Annex_IV_01_Efficacy sow study 1 Imasde.

[18]Annex_IV_02_Efficacy sow study 2 Tests and Trials.

[19]Annex_IV_03_Efficacy sows study 3 Imasde.

[20]Weight loss after farrowing = total litter weight × 1.465.

[21]RTQ_efficacy_02 and RTQ_efficacy_04_Statistical Output inc Batch Parity sow study 1

TABLE 3 Effects of Huvezym neXo on sows' performance and the apparent total tract digestibility (ATTD) of energy of the diets during gestation.

Trial	Groups (EPU-CU-XGU/kg feed)	Average daily feed intake Kg	Δ body weight Kg	Δ Backfat Mm	ATTD of energy (%)
1	0-0-0 1500-100-100	[redacted]	[redacted]	[redacted]	[redacted]
2	0-0-0 1500-100-100	[redacted]	[redacted]	[redacted]	[redacted]

Notes: Δ Body weight = body weight variation from the start of the trial until farrowing. Δ Backfat = backfat thickness variation from the start of the trial until entry to farrowing. The backfat thickness variation in trial 2 was recorded for the whole trial and is reported in Table 4.

Abbreviations: CU, glucanase units; EPU, xylanase units; XGU, xyloglucanase units.

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

TABLE 4 Effects of Huvezym neXo on sows' performance and the apparent total tract digestibility (ATTD) of energy of the diets during lactation.

Trial	Groups (EPU-CU-XGU/kg feed)	Average daily feed intake g	Δ body weight Kg	Δ Backfat Mm	ATTD energy (%)
1	0-0-0 1500-100-100	[redacted]	[redacted]	[redacted]	[redacted]
2	0-0-0 1500-100-100	[redacted]	[redacted]	[redacted]	[redacted]
3	0-0-0 1500-100-100	[redacted]	[redacted]	[redacted]	[redacted]

Notes: ATTD Energy = Apparent total tract digestibility of energy of the diet. Δ Body weight = body weight variation from farrowing until weaning. Δ Backfat = backfat thickness variation – in trials 1 and 2, it refers from the entry to farrow (Day 7 pre-farrowing) to weaning (26/21 days post-farrowing, respectively); in trial 3, the average daily feed intake and Δ backfat is for the whole experimental period (14 days pre-farrowing until weaning).

Abbreviations: CU, glucanase units; EPU, xylanase units; XGU, xyloglucanase units.

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

TABLE 5 Effects of Huvezym neXo on the performance of litter.

Trial	Groups (EPU-CU-XGU/kg feed)	Number of piglets born alive *n*	Number of piglets at weaning *n*	Piglet's body weight at birth Kg	Piglet's bodyweight at weaning Kg	Average daily gain g	Pre-weaning mortality and culls %
1	0-0-0 1500-100-100	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
2	0-0-0 1500-100-100	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
3	0-0-0 1500-100-100	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Abbreviations: CU, glucanase units; EPU, xylanase units; XGU, xyloglucanase units.

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

One sow of the control group died at farrow. The supplementation of the additive at the proposed use level resulted in higher sows' backfat loss during lactation (Table 4) and lower piglet BW at birth (Table 5). At weaning, the sows' backfat thickness ([redacted] mm for the control and supplemented group , respectively) and the individual piglet BW ([redacted] kg) were not statistically different between groups. Therefore, the Panel considered that the differences in backfat loss ([redacted]) during lactation and the BW at birth between groups were of little biological relevance. The sows receiving the additive at 1500 EPU, 100 CU and 100 XGU per kg showed a higher ATTD of energy during gestation and lactation (Tables 3 and 4).

In trial 2, [redacted]

[redacted][22]

Four sows were removed from the trial, three from the control group (one due to lameness and two others due to reproductive failure) and one from the supplemented group (due to reproductive failure). No effect of the additive supplementation was observed in any of the performance parameters recorded for sows or their litters, except for a higher feed intake of the sows during lactation. The inclusion of Huvezym neXo in sows' feed at 1500 EPU, 100 CU and 100 XGU per kg resulted in higher ATTD of energy during gestation. No differences were observed in the energy digestibility of the diets during lactation.

In trial 3, [redacted][23]

No sows died during the experiment. No differences in any of the performance parameters recorded for the sows or their litters were observed between groups. The inclusion of Huvezym neXo® in sows' feed at 1500 EPU, 100 CU and 100 XGU per kg resulted in higher ATTD of energy during lactation.

3.3.3 | Conclusions on efficacy

The FEEDAP Panel considers that the additive has the potential to improve the digestibility of the energy of the diets in gestating and lactating sows when supplemented at the proposed use level. This conclusion is extrapolated to all reproductive *Suidae*. Due to the lack of sufficient data, the Panel cannot conclude on the efficacy for other *Suidae* categories.

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[24] and Good Manufacturing Practice.

[22]RTQ_efficacy_07_Statistical output inc parity sow study 2

[23]RTQ_efficacy_02 and RTQ_efficacy_06_Statistical Output inc Batch Parity Sow study 3

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

4 | CONCLUSIONS

The information provided regarding the production strain allows confirming the taxonomic identification as *Trichoderma citrinoviride*.

The Panel notes that the final forms of the additive characterised, with a minimum enzyme activity of 15,000 EPU, 1000 CU and 1000 XGU per gram of product and an analytical ratio of xylanase:glucanase:xyloglucanase of ca. 7:1:1, do not comply with the enzyme ratio (15:1:1) of the specifications and of the test item used to perform the toxicological and tolerance studies. Therefore, the following conclusions apply exclusively to the product containing a minimum of 15,000 EPU, 1000 CU and 1000 XGU per gram and a xylanase:glucanase:xyloglucanase ratio of 15:1:1:

- The additive is safe for all *Suidae* at 1500 EPU, 100 CU and 100 XGU/kg complete feed.
- The use of the feed additive in feed for *Suidae* species is safe for the consumers and the environment.
- Huvezym® neXo 100 G is not an irritant to the skin and eyes but should be considered a skin sensitiser. Huvezym® neXo 100 L is not an irritant to the skin and eyes, and it is not a skin sensitiser. Both formulations of the additive are considered respiratory sensitisers.
- The additive has the potential to be efficacious in reproductive *Suidae* at the use level of 1500 EPU, 100 CU and 100 XGU/kg complete feed. Owing to the lack of sufficient data, the Panel cannot conclude on the efficacy of the additive for other *Suidae* categories.

No conclusions can be established on the safety of the final formulations of the feed additive as described in the characterisation section (enzyme ratio of ca. 7:1:1).

ABBREVIATIONS

ATTD	Apparent Total Tract Digestibility
BW	body weight
DM	dry matter
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
EURL	European Union Reference Laboratory
DSMZ	German Collection of Microorganisms and Cell Cultures GmbH
ITS	Internal Transcribed Spacers
NOAEL	no observed adverse effect level
RNA	Ribonucleic acid

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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.

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