

ADOPTED: 26 January 2022

doi: 10.2903/j.efsa.2022.7154

## **Efficacy of a feed additive consisting of endo-1,4-beta-xylanase produced by *Trichoderma citrinoviride* (IMI SD 135) (HOSTAZYM<sup>®</sup> X) for sows in order to have benefits in piglets (Huvepharma NV)**

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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 efficacy of HOSTAZYM<sup>®</sup> X as a feed additive for sows in order to have benefits in piglets. This additive consists of endo-1,4- $\beta$ -xylanase produced by a non-genetically modified strain of *Trichoderma citroviride*. The additive is to be used in sows at 1,500 EPU/kg feed. In a previous opinion, the FEEDAP Panel could not conclude on the efficacy of the additive when added to feed for sows in order to have benefits in piglets. The applicant provided new efficacy data to complete the assessment of the efficacy of the additive. Based on the previously assessed data and the newly submitted one, the Panel could not conclude on the efficacy of the additive.

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**Keywords:** zootechnical additives, digestibility enhancers, efficacy, xylanase, sows

**Requestor:** European Commission

**Question number:** EFSA-Q-2020-00396

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**Declarations of interest:** The declarations of interest of all scientific experts active in EFSA's work are available at <https://ess.efsa.europa.eu/doi/doiweb/doisearch>.

**Acknowledgments:** The Panel wishes to thank the following for the support provided to this scientific output: Working Group on Animal Nutrition.

**Suggested citation:** EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Durjava MF, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Dierick NA, Martelli G, Galobart J and Anguita M, 2022. Scientific Opinion on the Efficacy of a feed additive consisting of endo-1,4-beta-xylanase produced by *Trichoderma citrinoviride* (IMI SD 135) (HOSTAZYM® X) for sows in order to have benefits in piglets (Huvepharma NV). EFSA Journal 2022;20(3):7154, 9 pp. <https://doi.org/10.2903/j.efsa.2022.7154>

**ISSN:** 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.



## Table of contents

Abstract .....	1
1. Introduction .....	4
1.1. Background and Terms of Reference as provided by the requestor .....	4
1.2. Additional information .....	4
2. Data and methodologies .....	5
2.1. Data .....	5
2.2. Methodologies .....	5
3. Assessment .....	5
3.1. Efficacy .....	5
3.1.1. Conclusions on efficacy .....	8
3.2. Post-market monitoring .....	8
4. Conclusion .....	8
5. Documentation provided to EFSA/Chronology .....	8
References .....	9
Abbreviations .....	9

## 1. Introduction

### 1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003 establishes rules governing the Community authorisation of additives for animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Huvepharma NV, is seeking a Community authorisation of endo-1,4-beta-xylanase as a feed additive to be used as a gelling agent for sows,<sup>1</sup> in order to have benefit in piglets (Table 1).

**Table 1:** Description of the substances

<b>Category of additive</b>	Zootechnical additives
<b>Functional group of additive</b>	Digestibility enhancers
<b>Description</b>	Endo-1,4-beta-xylanase
<b>Target animal category</b>	Sows, in order to have benefit in piglets
<b>Applicant</b>	Huvepharma NV
<b>Type of request</b>	New opinion

On 27 November 2019,<sup>2</sup> the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the safety and efficacy of the product, could not conclude on the efficacy of endo-1,4-beta-xylanase in sows, in order to have benefit in piglets. After the discussion with the Member States on the Standing Committee, it was suggested to check for the possibility to demonstrate the efficacy.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of Authority's opinion. The new data have been received on 29 April 2020 and were already transmitted to the EFSA by the applicant.

In view of the above, the Commission asks the Authority to deliver a new opinion on endo-1,4-beta-xylanase as a feed additive for sows, in order to have benefit in piglets based on the additional data submitted by the applicant.

### 1.2. Additional information

The additive HOSTAZYM® X is a preparation of endo-1,4-beta-xylanase available in liquid and solid formulations. This product is authorised as a feed additive for chickens for fattening, turkeys for fattening, laying hens, minor poultry species for fattening and laying, weaned piglets and pigs for fattening,<sup>3</sup> chickens reared for laying and minor poultry species,<sup>4</sup> and carps.<sup>5</sup>

The FEEDAP Panel adopted two opinions on the safety and efficacy of the product as a feed additive for poultry and pigs (EFSA FEEDAP Panel, 2013, 2015), another one for its use as a feed additive in chickens reared for laying and minor poultry species reared for laying (EFSA FEEDAP Panel, 2017a), one on its use in feed for carps (EFSA FEEDAP Panel, 2017b) and the last one on the use as a feed additive in sows in order to have benefits in piglets (EFSA FEEDAP Panel, 2018a). In the latter, the FEEDAP Panel could not conclude on the efficacy of the product when added to feed for sows in order to have benefits in piglets.

<sup>1</sup> The mandate states to be used as a gelling agent, but the application was done for its use as a zootechnical additive as correctly stated in Table 1.

<sup>2</sup> It is noted that the date of adoption of the opinion is 2 October 2018.

<sup>3</sup> Commission implementing Regulation (EU) 2015/1043 of 30 June 2015 concerning the authorisation of the preparation of endo-1,4-beta-xylanase (EC 3.2.1.8) produced by *Trichoderma citrinoviride* Bisset (IM SD135) as a feed additive for chickens for fattening, turkeys for fattening, laying hens, weaned piglets, pigs for fattening and minor poultry species for fattening and for laying, and amending Regulations (EC) No 2148/2004, (EC) No 828/2007 and (EC) No 322/2009 (holder of authorisation Huvepharma NV). OJ L 167, 1.7.2015, p. 63.

<sup>4</sup> Commission implementing Regulation (EU) 2017/1906 of 18 October 2017 concerning the authorisation of a preparation of endo-1,4-b-xylanase (EC 3.2.1.8) produced by *Trichoderma citrinoviride* Bisset (IMI SD135) as a feed additive for chickens reared for laying and minor poultry species reared for laying (holder of authorisation Huvepharma NV). OJ L 269, 19.10.2017, p. 33.

<sup>5</sup> Commission implementing Regulation (EU) 2018/327 of 5 March 2018 concerning the authorisation of a preparation of endo-1,4-beta-xylanase (EC 3.2.1.8) produced by *Trichoderma citrinoviride* Bisset (IMI SD135) as a feed additive for carp (holder of authorisation Huvepharma NV). OJ L 63, 6.3.2018, p. 7.

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information<sup>6</sup> to a previous application on the same additive.<sup>7</sup>

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA.

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of HOSTAZYM® X (endo-1,4-beta-xylanase) is in line with the principles laid down in Regulation (EC) No 429/2008<sup>8</sup> and the Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b).

## 3. Assessment

The additive HOSTAZYM® X is a preparation of endo-1,4-beta-xylanase (xylanase; Enzyme Commission Number 3.2.1.8) produced by a non-genetically modified strain of *Trichoderma citrinoviride* (IMI SD 135). This additive is available in two solid and two liquid formulations. The solid formulations are HOSTAZYM® X 6000 MicroGranulate and 30000 MicroGranulate, which have a minimum guaranteed enzyme activity of 6,000 and 30,000 EPU<sup>9</sup>/g of product, respectively. The liquid formulations are HOSTAZYM® X 6000 Liquid and 15000 Liquid with a minimum guaranteed enzyme activity of 6,000 and 15,000 EPU/mL, respectively. The additive was characterised in full in EFSA's previous assessments (EFSA FEEDAP Panel, 2013, 2015).

The additive is intended to be used in feed for sows in order to have benefit in piglets at a recommended enzyme activity of 1,500 EPU/kg feed.

In a previous opinion (EFSA FEEDAP Panel, 2018a), the FEEDAP Panel could not conclude on the efficacy of the additive for sows. From the three trials in lactating sows considered in that assessment, positive effects of the additive at the recommended level were seen in only one study on the apparent total tract digestibility of the gross energy of the diets. In the present submission, the applicant has provided more data to support the efficacy of the product in sows in order to have benefit in piglets.

### 3.1. Efficacy

The applicant provided four new trials and a pooling of data including two of these new trials and two trials previously evaluated by the FEEDAP Panel (EFSA FEEDAP Panel, 2018a). One of the newly submitted studies<sup>10</sup> was rejected due to the discrepancies identified as regards to (i) the duration of the lactation of the piglets (19 vs. 21 days) and (ii) the methodologies followed in the last days of the lactation period. The discrepancies in the information given did not allow to know the correctness of the management of the animals and consequently may have had an impact on the suitability of the results obtained.

In Table 2, it is presented the trial design for the newly submitted studies (trials 1–3) and that of the studies previously evaluated but for which new data has been submitted (trials 4 and 5). In trial 1, sows were under study from service to the last day in lactation, while in the other trials considered, sows were under study from the last part of the gestation until weaning of the piglets and for a minimum duration of 28 days.

<sup>6</sup> FEED dossier reference: FAD-2020-0029.

<sup>7</sup> FEED dossier reference: FAD-2017-0062.

<sup>8</sup> 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.

<sup>9</sup> EPU: one EPU is the amount of enzyme which releases 0.0083 µmol of reducing sugars (xylose equivalent) per minute from oat spelt xylan at pH 4.7 and 50°C.

<sup>10</sup> Technical dossier FAD-2020-0029/Annex IV.03. The initial submission considered also a pooling of data of two studies, since one of the studies pooled was this rejected the study in which it was pooled the data was not further considered in the opinion., Annex IV.05.

**Table 2:** Design of the efficacy trials performed in sows

Trial	N per treatment	Breed (parity number)	Basal diet composition form	Groups (EPU/kg feed)		Duration of the study	
				Intended	Analysed*	Start	End
1 <sup>(a)</sup>	20 control 19 treated	Large White × Landrace (1–7)	Wheat, barley, rye and alfalfa (gestation) or soya bean meal (lactation) <i>pelleted</i>	0 1,500	190-220/200-210 1,810-1,460/ 1,800-1,730	Service	Weaning on day 28 of lactation
2 <sup>(b)</sup>	18	Piértrain × (Large White × Landrace × Tai Zumu) (1–7)	Wheat-soya bean meal in (gestation) or barley-corn-soya bean meal (lactation) <i>pelleted</i>	0 1,500	125/270 1,940/1,790	22 days prior to farrowing	Weaning on day 22 of lactation
3 <sup>(c)</sup>	20	Large White × Landrace (2–8)	Barley, wheat, wheat bran <i>pelleted</i>	0 1,500	300/670 1,820/2,440	21 days prior to farrowing	Weaning on day 21
4 <sup>(d)</sup>	16	Landrace × Large White (1–8)	Barley, corn, wheat, wheat middlings and soya bean	0 1,500	370 2,100	7 days before farrowing	Day 21 of lactation
5 <sup>(e)</sup>	15	Dutch Landrace × Dutch Large White (1–6)	Barley, wheat, wheat middlings and soya bean meal	0 1,500	310 2,200	7 days before farrowing	Day 27 of lactation

\*: In trials 1, 2 and 3 the values given are for gestation/lactation diets.

(a): Technical dossier FAD-2020-0029/Annex IV.02 and Supplementary information November 2021/Annexes RTQ2 and RTQ3.

(b): Technical dossier FAD-2020-0029/Annex IV.04.

(c): Technical dossier FAD-2020-0029/Supplementary information November 2021/Annex Q6.

(d): Technical dossier FAD-2017-0062/Supplementary information August 2018/Annex IV.02.

(e): Technical dossier FAD-2017-0062/Supplementary information August 2018/Annex IV.03 and IV.04.

In all trials, two experimental groups were considered which received either the basal diet not supplemented (control) or supplemented with HOSTAZYM® X at the minimum recommended level of 1,500 EPU/kg feed (confirmed by analysis). Farrowing performance was measured in all the trials (including at least the number of piglets born and their weight), along with sows' feed intake, body weight and back-fat loss during lactation. Except for trial 3, in all trials, it was measured the apparent faecal digestibility of the gross energy of the diets during gestation and/or lactation. Regarding the management of the litters, cross-fostering of piglets within treatment was done three days after farrowing in trial 1, up to 48 h after farrowing in trials 2, 3 and 5 or up to 24 h in trial 4. Piglets received creep feed in trials 1, 2, 3 and 5 from day 14, 10, 10 and 4 of life, respectively. The number of piglets, body weight and creep feed intake during lactation was recorded and the average daily weight gain was calculated to evaluate the growth of the litter.

In trial 1, the data were analysed by analysis of variance (ANOVA) with treatment as fixed effect, block (each block ideally consisted of at least 2 homogeneous sows) as the random effect and each sow with its respective litter was the experimental unit for all analyses. The number of piglets per litter at different time points and piglets' mortality were analysed with non-parametric methods (chi-squared test). The data of the other studies (2–5) were statistically analysed individually but these analyses are not described since the data from these studies was analysed after pooling.<sup>11</sup> At this regard, the data from studies 2–5 were pooled and analysed with mixed model for normally distributed data using the sow as the statistical unit and the trial as a random effect. Covariates were tested for inclusion as appropriate and retained in the model if they were significantly associated with the outcome. A manual stepwise backward selection procedure was used with a p-value for retention in the model as covariate

<sup>11</sup> Technical dossier FAD-2020-0029/Supplementary information November 2021/Annex RTQ 7.

of 0.15. The treatment variable was forced into the model, regardless of significance. It is noted that not all the variables were studied and that some of the parameters were calculated in a different way than in the original studies (e.g. feed intake is calculated for the overall period under study).

In trial 1, the results showed an improvement on the apparent total tract digestibility of the gross energy of the diets in the sows that received the additive, which would be considered as a positive effect of the additive. However, the sows that received the additive had lower body weight at weaning and showed a tendency ( $p = 0.063$ ) for higher body weight loss during lactation. The results observed on the weight of the sows would cast some doubts on the beneficial effect of the additive.

The results of trials 2–5, when considered singularly, did not show any relevant effect of the additive in the parameters measured. The analysis of the pooled data showed, however, that the piglets from sows receiving the additive had higher body weight at weaning ( $p = 0.051$ ) and significantly higher average daily weight gain during lactation ( $p = 0.045$ ) (Tables 3–4).

**Table 3:** Effect of HOSTAZYM® X on daily feed intake of the sows, body weight and condition, and faecal apparent digestibility of gross energy

Trial	Group (EPU/kg feed)	Daily feed intake in lactation (kg)*	Body weight (kg)			Back-fat loss (mm)	Apparent total tract digestibility of gross energy (%)****
			Farrowing**	Weaning	Weight variation***		
1	0	4.89	267	250 <sup>a</sup>	– 0.655	nd	78.4 <sup>B</sup> /80.8 <sup>b</sup>
	1,500	4.60	259	236 <sup>b</sup>	– 0.888	nd	80.1 <sup>A</sup> /81.9 <sup>a</sup>
2	0	6.00	235	237	+ 2.1	1.8	83.1/82.2
	1,500	5.90	237	237	+ 0.1	1.1	83.8/83.1
3	0	5.06	216	225	+ 8.8	0.7	–
	1,500	5.14	216	219	+ 3.3	1.0	–
4	0	5.32	252	216	– 36	2.4	74.5
	1,500	5.32	251	216	– 35	2.3	76.4
5	0	5.66	269	227	– 42	4.6	77.8
	1,500	5.66	270	225	– 45	5.0	78.0
Pooling of trials 2–5*****	0	4.75	–	–	– 24.3	2.31	–
	1,500	4.74	–	–	– 25.8	2.43	–

nd: not determined.

A,B: Within one trial and within a column, mean values with different superscript are different ( $p < 0.01$ ).

a,b: Within one trial and within a column, mean values with different superscript are different ( $p < 0.05$ ).

\*: Feed intake of the sows while kept in the lactation barn.

\*\* : Body weight of the sows is after farrowing in trial 1, at arrival at the farrowing barn for trials 4 and 5, and for trials 2 and 3 the sows were weighed prior to entering to the farrowing barn and the body weight after farrowing was estimated from the measured weight prior to farrowing and the contribution of fetuses, placenta and fluids.

\*\*\*: In trial 1, values are expressed per day; for the other trials, values represent the total variation for the lactation period (2 and 3) or overall study (4 and 5).

\*\*\*\*: Values in trials 1 and 2 correspond to gestation/lactation phases.

\*\*\*\*\*: Pooled data is for the overall study period.

**Table 4:** Effect of HOSTAZYM® X on litter size, piglets' weight and mortality during lactation

Trial	Group (EPU/ kg feed)	Litter size (n)		Piglets' weight (kg)		Individual daily weight gain (g)	Mortality/ culls***
		Initial*	Final	Initial**	Final		
1	0	12.7	11.6	1.49	6.95	207	1.7
	1,500	13.6	12.1	1.39	6.65	198	2.0
2	0	13.6	12.4	1.30	5.30	196	8.0
	1,500	13.1	11.8	1.30	5.70	216	10.0
3	0	11.9 <sup>a</sup>	9.7	1.47	5.78	205	18.5 <sup>a</sup>
	1,500	11.2 <sup>b</sup>	10.1	1.50	5.90	209	9.7 <sup>b</sup>
4	0	12.4	10.8	1.76 <sup>a</sup>	5.70	202	12.9
	1,500	12.8	10.9	1.51 <sup>b</sup>	5.67	212	14.4
5	0	13.6	12.7	1.21	7.31	231	10.8
	1,500	13.4	12.9	1.32	7.84	239	9.8
Pooling of trials 2–5****	0	13.3	11.5	1.50	5.90	200 <sup>b</sup>	–
	1,500	13.1	11.6	1.48	6.14	210 <sup>a</sup>	–

a,b: Within one trial and within a column mean values with different superscript are different ( $p < 0.05$ ).

\*: Initial litter size: trial 1 born alive in the other trials it is after cross-fostering.

\*\* : Initial body weight: after cross fostering in trials 1, 2, 3 and 4 or at birth in trial 5.

\*\*\*: In trial 1, values are piglets per sow after cross-fostering, in the other trials is per cent.

\*\*\*\*: Litter size at start means piglets born alive, initial body weight is after cross-fostering.

### 3.1.1. Conclusions on efficacy

In the previous opinion (EFSA FEEDAP Panel, 2018a), the FEEDAP Panel concluded that one trial showed a better apparent total tract digestibility of the gross energy of the diets in the lactating sows.

In the current assessment, the statistical analysis of pooled data from four studies showed improvements on the body weight and daily weight gain of the piglets during lactation, and this would be considered as one positive outcome.

Overall, there is not sufficient data to conclude on the efficacy of the additive for sows during lactation or in order to have benefit in piglets.

### 3.2. 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<sup>12</sup> and Good Manufacturing Practice.

## 4. Conclusion

The FEEDAP Panel cannot conclude on the efficacy of the additive in sows in order to have benefits in piglets.

## 5. Documentation provided to EFSA/Chronology

Date	Event
<b>20/04/2020</b>	Dossier received by EFSA. HOSTAZYM® X for sows in order to have benefits in piglets. Submitted by Huvepharma N.V.
<b>19/05/2020</b>	Reception mandate from the European Commission
<b>14/09/2020</b>	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: Efficacy</i>
<b>16/11/2021</b>	Reception of supplementary information from the applicant - Scientific assessment re-started
<b>26/01/2022</b>	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

<sup>12</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.



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## Abbreviations

- ANOVA analysis of variance  
FEEDAP EFSA Panel on Additives and Products or Substances used in Animal