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## Safety and efficacy of HOSTAZYM<sup>®</sup> X (endo-1,4-beta-xylanase) as a feed additive for rabbits for fattening

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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 HOSTAZYM<sup>®</sup> X as a feed additive for rabbits for fattening. HOSTAZYM<sup>®</sup> X contains endo-1,4-beta-xylanase produced by a strain of *Trichoderma citrinoviride* and is available in liquid and solid formulations. It is authorised as a feed additive for chickens for fattening, turkeys for fattening, laying hens, minor poultry species for fattening and laying, weaned piglets, pigs for fattening and carps. The applicant has now requested to extend the authorisation to rabbits for fattening. The FEEDAP Panel considered that the new use of the additive would not change the previous conclusions regarding the safety for the consumer, user and environment. There are no concerns for consumer safety and no risks for the environment are expected from the use of the additive as a feed additive. Regarding the safety for the user, the additive should be considered a potential skin and eye irritant, and a potential skin and respiratory sensitiser. A subchronic oral toxicity study in rats was submitted to support the safety of the additive when administered to rabbits for fattening. The maximum safe enzyme activity in feed for the rabbits for fattening was derived from the data obtained in the subchronic oral toxicity study in rats; considering the maximum safe intake derived for rabbits from the toxicological data, the FEEDAP Panel concluded that the additive is safe for rabbits for fattening at the recommended use level. Two efficacy studies were submitted to support the efficacy of the additive; a significant effect of the additive in a relevant parameter was found in only one trial. Due to the insufficient evidence the Panel could not conclude on the efficacy of the additive in rabbits for fattening.

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## 1. Introduction

### 1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003<sup>1</sup> 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 Huvepharma EOOD<sup>2</sup> for authorisation of the product HOSTAZYM® X (endo-1,4-beta-xylanase), when used as a feed additive for rabbits for fattening (category: zootechnical additive; 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 6 August 2018.

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 HOSTAZYM® X (endo-1,4-beta-xylanase), when used under the proposed conditions of use (see Section 3.1).

### 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 another one regarding its use in sows in order to have benefits in piglets (EFSA FEEDAP Panel, 2018).

## 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<sup>6</sup> in support of the authorisation request for the use of HOSTAZYM® X (endo-1,4-beta-xylanase) as a feed additive for rabbits for fattening.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.<sup>7</sup>

<sup>1</sup> 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.

<sup>2</sup> Huvepharma EOOD, Uitbridingstraat 80, 2600 Antwerp, Belgium.

<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 (IMI 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.

<sup>6</sup> FEED dossier reference: FAD-2018-0025.

<sup>7</sup> The full report is available on the EURL website: <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2017-0010>

## 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 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, 2011) and Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c).

## 3. Assessment

The additive HOSTAZYM® X is a preparation of endo-1,4-beta-xylanase intended to be used as a zootechnical additive (functional group: digestibility enhancers) in feed for rabbits for fattening.

### 3.1. Characterisation

The endo-1,4-beta-xylanase (xylanase; EC 3.2.1.8) present in this additive is produced by a non-genetically modified strain of *Trichoderma citrinoviride* Bisset (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>8</sup>/g of product, respectively. The liquid formulations are HOSTAZYM® X 6000 Liquid and 15,000 Liquid with a minimum guaranteed enzyme activity of 6,000 and 15,000 EPU units/mL. The additive as well as the production strain were characterised in full in the previous assessments (EFSA FEEDAP Panel, 2013, 2017a).

The applicant submitted new data on the stability and the capacity to homogeneously distribute of the xylanase in feed for rabbits. Three batches of HOSTAZYM® X 30000 MicroGranulate were added to a complete feed for rabbits for fattening to provide 1,500 EPU/kg feed.<sup>9</sup> Samples of the mash feeds were heat treated (85°C), and the effect of the heat treatment on the enzyme was studied. The capacity of the xylanase to homogeneously distribute was studied in 10 subsamples for each type of feed (mash and pelleted) and the coefficient of variation was calculated. The stability of the xylanase to storage was studied after storage of mash and pelleted feed at 25°C and 40°C for 3 months. The mean enzyme activity in mash feed was 1,700 EPU/kg feed and the enzyme activity showed a coefficient of variation of 11% in the samples analysed. Recovery of xylanase activity after heat treatment was of 95–97%. The recovery of xylanase after storage of mash and pelleted samples was > 92% for samples stored at 25°C and > 88% for those stored at 40°C.

The additive is to be used in feed for rabbits for fattening at a recommended enzyme activity of 1,500 EPU/kg feed.

### 3.2. Safety

Safety aspects regarding the use of this additive in feed including the safety for the consumers, for the users and for the environment have been previously evaluated (EFSA FEEDAP Panel, 2013, 2015). The FEEDAP Panel concluded that there are no concerns for the consumer safety and no risks for the environment are expected. As regards the safety for the user, it was concluded that the additive should be considered a potential skin and eye irritant, and a potential skin and respiratory sensitiser. The FEEDAP Panel is not aware of any new information that would lead to reconsider the conclusions drawn previously. Moreover, the FEEDAP Panel considers that the new use requested by the applicant would not introduce hazards/risks not considered in previous assessments.

Since the application covers the use of the additive in rabbits for fattening, the safety for this new target species needs to be assessed.

#### 3.2.1. Safety for rabbits for fattening

In order to support the safety for the target species, the applicant submitted a subchronic oral toxicity study in rats.<sup>10</sup> This subchronic oral toxicity study has been previously evaluated by the FEEDAP Panel (EFSA FEEDAP Panel, 2015). The no-observed-adverse-effect-level (NOAEL) was

<sup>8</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 4.5 and 50°C.

<sup>9</sup> Technical dossier/Section II/Annex II.57.

<sup>10</sup> Technical dossier/Section III/Annexes 2 and 3.

established to be 1,000 mg test item/kg body weight and day. The test item was the concentrate that is used to prepare the additive which contains 1,000 EPU/mg test item; consequently, the NOAEL in enzyme activity units would correspond to 1,000,000 EPU/kg body weight and day.

The maximum safe enzyme activity in feed for rabbits for fattening was derived following EFSA FEEDAP Panel's guidance on the safety for the target species (EFSA FEEDAP Panel, 2017c). To this scope, the NOAEL of 1,000,000 EPU/kg body weight was retained, an uncertainty factor of 100 was applied and a default value of daily feed intake of the rabbits of 50 g dry matter/kg body weight and day was retained. The content of dry matter in complete feed is assumed to be 88%. The resulting maximum safe intake for rabbits for fattening was of 176,000 EPU/kg feed.

The recommended dose for rabbits for fattening is of 1,500 EPU/kg feed. Considering the maximum safe intake derived for rabbits for fattening from a toxicological study, the FEEDAP Panel concludes that the additive is safe for rabbits for fattening at the recommended use level.

### 3.3. Efficacy for rabbits for fattening

Two efficacy trials were submitted by the applicant. In the first trial, a total of 60 weaned male rabbits (California × Hybrid, 28 days of life) individually caged received four dietary treatments (resulting in 15 replicates per group).<sup>11</sup> A basal diet based on alfalfa, wheat middlings, soybean hulls, beet pulp and sunflower meal was either not supplemented (control diet), or supplemented with HOSTAZYM® X (endo-1,4-beta-xylanase) to provide 1,050, 1,500 or 3,000 EPU/kg feed (confirmed by analysis). The feed was offered to the rabbits in pelleted form *ad libitum* for a total of 42 days.

In the second trial, done in a commercial farm, a total of 720 male and female weaned rabbits (new Zealand White, 28 days of life) were caged in groups of 8 rabbits and distributed to three dietary treatments (resulting in 30 replicates per group).<sup>11</sup> A basal diet based on alfalfa, wheat bran starch, sunflower meal, barley and beet and apple pulp was either not supplemented (control diet) or supplemented with HOSTAZYM® X (endo-1,4-beta-xylanase) to provide 1,050 or 1,500 EPU/kg feed. Analytical results showed enzyme activities of 240, 1,750 and 2,220 EPU/kg feed for the control diet and diets containing 1,050 or 1,500 EPU/kg feed, respectively. The feed was offered to the rabbits in pelleted form and on *ad libitum* basis for a total of 42 days.

In the two studies, the animal health status and mortality were monitored daily. The animals were weighed and feed intake was measured at different stages of the study. Feed to gain ratio was calculated for the corresponding periods. In trial 1, faeces were collected from 10 replicates per treatment from day 35 to 39 and analysed for digestibility of the dry matter and fibre fractions<sup>12</sup> (total collection method). An analysis of variance was done with the data (the pen as the experimental unit). The results of the studies are presented in Table 1.

**Table 1:** Effects of HOSTAZYM® X on the performance of rabbits for fattening (from weaning to day 42)

Trial	Groups (EPU/kg feed)	Daily feed intake (g)	Body weight (g)		Feed to gain ratio	Mortality (%)
			Initial	Final		
1	0	129	861	2,541	3.33	13.3
	1,050	128	814	2,557	3.32	6.7
	1,500	123	830	2,533	3.17	6.7
	3,000	135	796	2,572	3.41	0
2	0	129	593	2,109	3.95 <sup>X</sup>	12.9
	1,050	126	595	2,119	3.63 <sup>XY</sup>	8.3
	1,500	125	596	2,132	3.58 <sup>Y</sup>	7.3

X,Y: Values in the same column and for the same trial with different superscript are different 0.05 < p < 0.10.

In trial 1, no statistical differences were observed in any of the zootechnical parameters measured in the rabbits. Results showed no modifications on the digestibility of dry matter but groups receiving the enzyme had increased the digestibility of some of the fractions of the fibre. However, the utilisation of the energy (e.g. metabolisable energy or energy retention by carcass analysis), which is considered

<sup>11</sup> Technical dossier/Section IV/Annex IV.1.

<sup>12</sup> Including neutral and acid detergent fibre, acid detergent lignin, hemi/cellulose, total dietary fibre, soluble fibre, non-soluble fibre, total non-starch polysaccharides.

as the relevant parameter to demonstrate the efficacy of the enzyme, was not studied. Therefore, this study did not provide evidence of efficacy of the additive.

In trial 2, the mortality – including culling of the rabbits during the study – was 13%, 8% and 7% for control, 1,050 and 1,500 groups, respectively. The causes and time of mortality/culling were not reported. No statistical differences were found on the daily feed intake or the body weight of the rabbits; however, the group receiving 1,500 EPU/kg feed showed a significantly better feed to gain ratio (3.58 vs 3.95). Therefore, this trial shows a significant and positive effect of the additive on the feed to gain ratio of the rabbits.

In summary, significant and positive effects of the additive were observed in only one trial. Consequently, the Panel cannot conclude on the efficacy of the additive in rabbits for fattening due to the insufficient evidence.

### 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<sup>13</sup> and Good Manufacturing Practice.

## 4. Conclusions

HOSTAZYM® X is safe for the consumer and no risks for the environment are expected when used as a feed additive for rabbits for fattening. The additive should be considered a potential skin and eye irritant, and a potential skin and respiratory sensitiser.

HOSTAZYM® X is safe for rabbits for fattening at 1,500 EPU/kg feed. However, the Panel cannot conclude on the efficacy of the additive for rabbits for fattening due to the insufficient evidence provided.

## Documentation provided to EFSA

- 1) HOSTAZYM® X for rabbits for fattening. July 2018. Submitted by Huvepharma EOOD.
- 2) Comments from Member States.

## Chronology

Date	Event
3/5/2018	Dossier received by EFSA
18/5/2018	Reception mandate from the European Commission
6/8/2018	Application validated by EFSA – Start of the scientific assessment
6/11/2018	Comments received from Member States
27/11/2018	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

## References

- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Technical guidance: tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. <https://doi.org/10.2903/j.efsa.2011.2175>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. <https://doi.org/10.2903/j.efsa.2012.2536>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of HOSTAZYM X (endo-1,4-beta-xylanase) as a feed additive for poultry, piglets and pigs for fattening. EFSA Journal 2013;11(2):3105, 23 pp. <https://doi.org/10.2903/j.efsa.2013.3105>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific Opinion on the safety of HOSTAZYM X as a feed additive for poultry and pigs. EFSA Journal 2015;13(1):3969, 10 pp. <https://doi.org/10.2903/j.efsa.2015.3969>

<sup>13</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.

- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2017a. Safety and efficacy of HOSTAZYM® X (endo-1,4- $\beta$ -xylanase) as a feed additive for chickens reared for laying and minor poultry species reared for laying. EFSA Journal 2017;15(2):4708, 6 pp. <https://doi.org/10.2903/j.efsa.2017.4708>
- EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), 2017b. Scientific Opinion on the safety and efficacy of HOSTAZYM® X (endo-1,4-b-xylanase) as a feed additive for carps. EFSA Journal 2017;15(7):4942, 9 pp. <https://doi.org/10.2903/j.efsa.2017.4942>
- EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), 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), 2018. Scientific opinion on the safety and efficacy of Hostazym® X (endo-1,4-beta-xylanase) as a feed additive for sows in order to have benefit in piglets. EFSA Journal 2018;16(10):5455, 8 pp. <https://doi.org/10/2903/j.efsa.2018.5456>.

## Abbreviations

EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances in Animal Feed
NOAEL	no-observed-adverse-effect-level