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Safety and efficacy of HOSTAZYM[®] X (endo-1,4- β -xylanase) as a feed additive for carps

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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[®] X. The additive HOSTAZYM[®] X is a preparation of endo-1,4- β -xylanase available in different 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. The FEEDAP Panel adopted two opinions on the safety and efficacy of the product as a feed additive for poultry and pigs, and another one for its use as a feed additive in chickens reared for laying and minor poultry species reared for laying. The applicant has now requested to extend the authorisation to carps (*Cyprinus carpio*). 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. The FEEDAP Panel concluded that there are no concerns for consumer safety and no risks for the environment are expected. Considering 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 data in a tolerance trial performed with carps showed that the additive is safe for carp at 1,050 endo-pentosanase units (EPU)/kg feed. The results in three efficacy trials showed that the additive has the potential to improve the performance of the carps at 1,050 EPU/kg feed.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ 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 EOOD² for authorisation of the product HOSTAZYM® X (endo-1,4- β -xylanase), when used as a feed additive for carps (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 10 April 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 HOSTAZYM® X (endo-1,4- β -xylanase), when used under the proposed conditions of use (see Section 3).

1.2. Additional information

The additive HOSTAZYM® X is a preparation of endo-1,4- β -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.³

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) and another one for its use as a feed additive in chickens reared for laying and minor poultry species reared for laying (EFSA FEEDAP Panel, 2017). The applicant is now requesting for an extension of use of the additive to carps.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁴ in support of the authorisation request for the use of HOSTAZYM® X (endo-1,4- β -xylanase) as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008⁵ and the applicable EFSA guidance documents.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁶

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 Huvepharma EOOD, 3a Nikolay Haytov Str, 1113 Sofia, Bulgaria.

³ 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 SD 135) 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, pp. 63–66.

⁴ FEED dossier reference: FAD-2017-0010.

⁵ 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. 1–65.

⁶ The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2017-0010_endo-1-4-beta-xylanase_4a1617.pdf

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of HOSTAZYM® X (endo-1,4- β -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) and Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011).

3. Assessment

This assessment deals with a request from the applicant to extend the use of HOSTAZYM® X in feed for carps at a recommended dose of 1,050 EPU/kg feed.

3.1. Characterisation

The additive HOSTAZYM® X is a preparation of endo-1,4- β -xylanase (xylanase; EC 3.2.1.8) 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 6,000 MicroGranulate and 30,000 MicroGranulate, which have a minimum guaranteed enzyme activity of 6,000 and 30,000 EPU⁷ /g of product, respectively. The liquid formulations are HOSTAZYM® X 6,000 Liquid and 15,000 Liquid with a minimum guaranteed enzyme activity of 6,000 and 15,000 EPU/mL. The additive was characterised in full in the previous assessments (EFSA FEEDAP Panel, 2013, 2017).

The applicant provided new data on the stability and the capacity of homogeneously distribute of the xylanase from HOSTAZYM® X 6000 Liquid in feed for fish (Nile tilapia, *Oreochromis niloticus*).⁸ Three batches of the additive were added to the feed at three different supplementation dosages 1,050, 1,500 or 3,000 EPU/kg feed. The addition of the enzyme was done after extrusion of the feeds. The enzyme was applied together with oil by coating under vacuum. Samples of the feed were stored at three different temperatures, 15°C and 25°C up to 12 weeks or at 37°C for up to 16 weeks. After 12 weeks, the recovery of the xylanase in the feed stored at 15°C was higher than 90% in all cases, at 25°C and 37°C was similar to or above 80%. In samples kept at 37°C, the recovery values after 16 weeks were below 70%. After the preparation of the feeds, seven samples from each were analysed to investigate the capacity to homogeneously distribute of the xylanase. The coefficient of variation of the xylanase activity in the feeds was below 9% in all cases (3–9%).

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 has 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. Considering 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 it to reconsider the conclusions drawn previously. Moreover, the FEEDAP Panel considers that the new use requested by the applicant would not modify the above conclusions.

Since the application covers the use of the additive in fish, the applicant provided one tolerance study in common carp (*Cyprinus carpio*).

3.2.1. Safety for the target species

In a combined tolerance and efficacy trial, a total of 720 juvenile common carp (*Cyprinus carpio*) were reared in standard conditions for approximately 7 weeks. At that point, with a body weight of 13.6 ± 0.6 g, fish were distributed in groups of 30–24 tanks and allocated to six dietary treatments (representing four replicate tanks per treatment).⁹ A basal diet based on fish meal (8.5%), porcine blood meal (10%), soybean meal (13%), Brewer's yeast (6%), rapeseed meal (10%) and wheat meal (41.6%) (crude protein 33%, crude fat 7.3% and gross energy 18.1 MJ/kg feed) was either not supplemented (control) or supplemented with HOSTAZYM® X 6,000 Liquid to provide endo-1,4- β -

⁷ EPU: one EPU is the amount of enzyme which releases 0.0083 μ mol of reducing sugars (xylose equivalent) per minute from oat spelt xylan at pH 4.7 and 50°C.

⁸ Technical dossier/Section II/Annex II.51.

⁹ Technical dossier/Section III/Ref_III_1_TAS.

xylanase at 1,050 (1×), 1,500, 3,000 or 300,000 (285× the maximum recommended dose) EPU/kg feed. The xylanase activity was confirmed by analysis. A positive control (fish meal (13.5%) increased in expense of wheat meal 37.1%, crude protein 35.2%, crude fat 7.3% and gross energy 18.6 MJ/kg feed) was also included. Pelleted feed was hand fed avoiding feed wastage for 90 days. Temperature in the tanks was $27.1 \pm 0.1^\circ\text{C}$ and dissolved oxygen levels were kept above 6.1 mg/L. Survival and health status of fish were monitored. Feed intake was recorded and fish were weighed individually on day 0 and group weighed on days 45 and 90 of the experiment. From the data, specific growth rate (% body weight per day), feed to gain ratio, relative daily feed intake (% of body weight per day) and protein efficiency ratio were calculated. At the end of the study (day 91), eight fish per tank (32 fish per treatment) were killed, individually weighed, externally examined and then dissected for gross pathology examination of the following organs: skin, gills, spleen, head kidney, viscera and liver. The liver of these fish was weighed. An analysis of variance (ANOVA) was done with the data obtained and group means were compared with the Student–Newman–Keuls test. Differences were considered significant at a level of at least $p < 0.05$.

No fish died during the study and no signs of fish distress were observed throughout the experiment. Final body weight of the fish was 59.8, 75.6, 87.7, 91.3, 100.8 and 90.8 g for the control, 1,050, 1,500, 3,000 and 300,000 EPU/kg feed and positive control, respectively, and the corresponding values for the feed to gain ratio were 1.34, 1.18, 1.10, 1.08, 1.08 and 1.12 (for details see Table 1 in Section 3.3). The body weight of the fish was significantly higher and the feed to gain ratio was lower with the xylanase at any dose (from 1,050 EPU/kg feed) compared to the control group. No abnormal findings were reported from gross pathology.

The supplementation of the experimental diets with HOSTAZYM® X 6,000 Liquid at up to 285× the maximum recommended dose did not have any negative effect on the performance and health of juvenile carp. Therefore, the additive is considered safe for carps at the proposed dose (1,050 EPU/kg feed).

3.3. Efficacy

Three trials performed in three different countries were submitted.

The first efficacy trial was the tolerance trial,¹⁰ already described in Section 3.2.

In the second efficacy trial, a total of 720 juvenile Jian carp (*Cyprinus carpio* var. Jian) were reared in standard conditions for approximately 4 weeks. At that point and with an initial body weight of 7.99 ± 0.01 g, fish were distributed in groups of 30–24 tanks and allocated to four dietary treatments (representing six replicate tanks per treatment).¹¹ A basal diet based on fish meal (7%), soybean meal (15%), rice gluten meal (10.5%), cottonseed meal (15%), rapeseed meal (10%) and wheat middlings (31.1%) (crude protein 34% and crude fat 6.9% feed) was either not supplemented (control) or supplemented with HOSTAZYM® X 6,000 MicroGranulate to provide xylanase at 1,050, 1,500 or 1,950 EPU/kg feed. The xylanase activity was confirmed by analysis. Pelleted feed was offered by hand feeding avoiding feed wastage for 70 days. Temperature in the tanks was 24–26°C and dissolved oxygen levels were kept above 5 mg/L.

The third trial was performed with a total of 240 two-year-old common carp (*Cyprinus carpio*) with an initial body weight of approximately 375 g. Fish were distributed in groups of 20 fish to 12 ponds and allocated to four dietary treatments (representing three replicate ponds per treatment).¹² A basal diet based on fish meal (8.5%), erythrocyte meal (10%), yeast (6%), soybean meal (13%), rapeseed meal (10%) and wheat meal (46%) (crude protein 33%, crude fat 7.1% and gross energy 18.0 MJ/kg feed) was either not supplemented (control) or supplemented with HOSTAZYM® X 6,000 Liquid to provide endo-1,4-β-xylanase at 1,050, 1,500 or 3,000 EPU/kg feed. The xylanase activity was confirmed by analysis. Pelleted feed was fed with an automatic band feeder avoiding feed wastage for 90 days. Temperature in the ponds ranged from 13.5°C to 24.7°C and dissolved oxygen levels ranged from 2.0 to 5.1 mg/L.

In all trials, survival of the animals was monitored, feed distributed and body weight was measured throughout the study and specific growth rate, feed to gain ratio and relative daily feed intake were calculated. Other measurements including apparent digestibility, intestinal microbiota and body composition were performed.

¹⁰ Technical dossier/Section IV/Ref_IV_2_efficacy 3.

¹¹ Technical dossier/Section IV/Ref_IV_1_efficacy 1.

¹² Technical dossier/Section IV/Ref_IV_2_efficacy 2.

In all the trials, an analysis of variance was performed on the data obtained and mean values were compared with the Student–Newman–Keuls test (trial 1), Duncan’s test (trial 2) and Tukey’s test (trial 3). The experimental unit was the tank. Differences were considered significant at a level of at least $p < 0.05$.

Results are shown in Table 1. In all trials, performance was improved by the additive at the lowest tested dose (at 1,050 EPU/kg feed). Therefore, the FEEDAP Panel concludes that the additive has the potential to improve the performance of carps at 1,050 EPU/kg, in diets where the substrate for the xylanase is present.

Table 1: Effects of HOSTAZYM® X on feed intake, final body weight, feed to gain ratio and specific growth rate of carps

Trial	Groups (EPU/kg feed)	Feed intake ¹	Final body weight (g)	Feed to gain ratio	Specific growth rate (% body weight/day)
1	Control	1.87 ^b	59.8 ^a	1.34 ^c	1.64 ^a
	1,050	1.82 ^{ab}	75.6 ^b	1.18 ^b	1.90 ^b
	1,500	1.78 ^a	87.7 ^c	1.10 ^a	2.07 ^c
	3,000	1.78 ^a	91.3 ^c	1.08 ^a	2.12 ^c
	300,000	1.83 ^{ab}	100.8 ^d	1.08 ^a	2.22 ^d
2	Control	116 ^a	80.4 ^a	1.60 ^c	3.15 ^a
	1,050	132 ^{bc}	115.5 ^c	1.23 ^a	3.71 ^c
	1,500	134 ^c	118.6 ^c	1.21 ^a	3.75 ^c
	1,950	130 ^b	106.6 ^b	1.32 ^b	3.59 ^b
3	Control	26.7 ^a	1,191 ^a	1.61	1.31
	1,050	28.8 ^b	1,233 ^b	1.65	1.33
	1,500	28.9 ^b	1,236 ^b	1.63	1.34
	3,000	29.0 ^b	1,276 ^b	1.62	1.32

EPU: endo-pentosanase units.

1: In trial 1, feed intake values are expressed in % body weight per day, in trial 2, values are total feed intake per fish in grams, and in trial 3, values are total feed intake per replicate in kg.

a,b,c,d Values within a trial and within a column with a different superscript are significantly different ($p < 0.05$).

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 Regulation¹³ and Good Manufacturing Practice.

4. Conclusions

In previous opinions, the FEEDAP Panel concluded that there are no concerns for consumer safety and no risks for the environment are expected and considers that the new use of HOSTAZYM® X would not modify these conclusions. Considering 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 additive is safe and has the potential to improve the performance of carps (*Cyprinus carpio*) at 1,050 EPU/kg feed.

Documentation provided to EFSA

- 1) HOSTAZYM® X for carps. February 2017. Submitted by Huvepharma EOOD.
- 2) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for HOSTAZYM® X.
- 3) Comments from Member States.

¹³ 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.

References

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- 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>
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Abbreviations

ANOVA	analysis of variance
EC	Enzyme Commission
EPU	endo-pentosanase units
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for HOSTAZYM X

In the current application, authorisation is sought under Article 4(1) for endo-1,4- β -xylanase produced by *Trichoderma citrinoviride* Bisset (IM SD135) (4a1617), under the category/functional group 4 (a) 'zootechnical additive'/digestibility enhancers', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for carp. Endo-1,4- β -xylanase (EC 3.2.1.8) has been already authorised as a feed additive under Commission Implementing Regulation (EU) 2015/1043. The activity of endo-1,4- β -xylanase is expressed as endo-pentosanase units (EPU). According to the Applicant, one EPU is the amount of enzyme which releases 0.0083 μ mol of reducing sugars (xylose equivalent) per minute from oat spelt xylan at pH 4.7 and 50°C. The feed additive is intended to be marketed in solid or liquid formulations (HOSTAZYM® X) with a guaranteed minimum activity of endo-1,4- β -xylanase ranging from 6,000 to 30,000 EPU/g. The formulated feed additive is proposed to be incorporated via premixtures or directly into feedingstuffs with a minimum recommended endo-1,4- β -xylanase activity of 1,050 EPU/kg complete feed. For the quantification of the endo-1,4- β -xylanase activity in the feed additive, premixtures and feedingstuffs, the Applicant submitted spectrophotometric methods based on the quantification of water-soluble dyed fragments produced at pH 4.7 and 50°C by the action of endo-1,4- β -xylanase on commercially available azurine cross-linked wheat arabinoxylan substrates from Megazyme. The analytical methods presented were already evaluated in the frame of the authorised dossier FAD-2010-0001. Furthermore, the Applicant submitted a verification study demonstrating the fitness for purpose of the analytical method for the quantification of endo-1,4- β -xylanase in fish feed. The following performance characteristics were derived from the data presented: a relative standard deviation for repeatability (RSDr) and for intermediate precision (RSDip) ranging from 1.9% to 16%; and a recovery rate (RRec) ranging from 93% to 116%. Based on the satisfactory performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified colorimetric methods submitted by the Applicant for the quantification of endo-1,4- β -xylanase in the feed additive, premixtures and feedingstuffs. Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.