SCIENTIFIC OPINION

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Safety and efficacy of Hemicell® HT (endo-1,4-β-mannanase) as a feed additive for chickens for fattening, chickens reared for laying, turkey for fattening, turkeys reared for breeding, weaned piglets, pigs for fattening and minor poultry and porcine species

EFSA Panel on Additives and Products or Substances used in Animal Feed (EFSA FEEDAP Panel),
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Abstract

Hemicell® HT/HT-L is an additive that presents endo-1,4-β-mannanase produced by a genetically modified strain of *Paenibacillus lentus.* This additive is aimed to be used as a feed additive for chickens for fattening/reared for laying, turkeys for fattening/reared for breeding, weaned piglets, pigs for fattening and minor poultry and porcine species. In a previous assessment, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) established the safety of the additive regarding the production strain, target species, consumer and user. However, limitations on the data regarding the absence of the production strain and its DNA did not permit to conclude on the safety of the additive for the environment. In that assessment, the Panel considered that the additive has a potential to be efficacious in the target species with the exception of pigs for fattening and in minor porcine species. The applicant provided new data to address the limitations identified. New data provided allowed the FEEDAP Panel to conclude that production strain and its recombinant DNA are not detected in the intermediate product used to formulate the additive. Therefore, the Panel concluded that the additive does not pose any environmental safety concern. Regarding the efficacy in pigs for fattening, the applicant provided a new analysis of the trials previously assessed which allowed the Panel to conclude that the additive has a potential to improve the feed to gain ratio at 32,000 U/kg feed. No data was provided regarding the efficacy in minor porcine species. However, considering the data available in major porcine species and that the mode of action of enzymes is well-known and can be reasonably be considered to be similar among porcine species the Panel extrapolated the conclusion drawn in pigs for fattening to minor porcine species for fattening.

Keywords: zootechnical additives, digestibility enhancers, safety, efficacy, endo-1,4-β-mannanase, poultry, pigs

Requestor: European Commission

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Table of contents

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 Eli Lilly and Company Ltd, is seeking a Community authorisation of Endo-1,4-β-mannanase, EC 3.2.1.78 to be used as a zootechnical additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, weaned piglets, pigs for fattening and minor poultry/porcine species (Table 1)

Table 1: Description of the substances

Category of additive	Zootechnical additive
Functional group of additive	Digestibility enhancers
Description	Endo-1,4-β-mannanase, EC 3.2.1.78
Target animal category	Chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, weaned piglets, pigs for fattening and minor poultry/porcine species
Applicant	Eli Lilly and Company Ltd
Type of request	New opinion

On 07 December 2016, 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 environmental safety of the product with regard to the genetically modified production strain. The Panel could also not conclude on the efficacy of the product in pigs for fattening and in minor porcine species.

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 sent to EFSA and Commission on 20 December 2017.

In view of the above, the Commission asks the Authority to deliver a new opinion of endo-1,4-β-mannanase, EC 3.2.1.78 as a zootechnical additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, weaned piglets, pigs for fattening and minor poultry/porcine species based on the additional data submitted by the applicant.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of Supplementary information[1] to a previous application.[2]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Hemicell® HT (endo-1,4-β-mannanase) 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), Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008) and Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011).

3. Assessment

The additive Hemicell® HT/HT-L contains endo-1,4-β-mannanase (Enzyme Commission number: 3.2.1.78, mannanase) as the main enzyme activity and is available in two forms, solid (Hemicell® HT) and liquid (Hemicell® HT-L). The mannanase enzyme is produced by a genetically modified strain of

[1] FAD-2017-0070.
[2] FAD-2014-0001.

Paenibacillus lentus (formerly named *Bacillus lentus*), which is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ) with the accession number DSM 28088.

The production strain and the additive were fully characterised in a previous assessment (FEEDAP Panel, 2017). In that assessment, the safety of this additive for the target species and consumers were established. Regarding the safety for the user the Panel concluded that Hemicell® HT and Hemicell® HT-L are not irritant to the skin and eyes, Hemicell® HT is a skin sensitiser and the additive is considered a potential respiratory sensitiser. However, the Panel could not conclude on the environmental safety of the product because uncertainty remained regarding the presence of the production strain and its DNA in the product. The Panel also concluded that the additive has a potential to be efficacious in the target species with the exception of pigs for fattening and minor porcine species for fattening, for which no conclusion could be drawn.

The applicant has now provided new data/information regarding the presence of cells and DNA of the production strain and the efficacy in pigs.

3.1. Characterisation of the additive

Hemicell® HT, including aspects of the production strain, has been characterised in a previous opinion (EFSA FEEDAP Panel, 2017). In that assessment, the applicant provided data on the absence of the production strain and its DNA in the intermediate product that is used to formulate the additive. Owing to the methodologies followed, uncertainty remained on the presence of the production strain and its recombinant DNA in the additive. Additional data have been submitted on the absence of the production strain and its recombinant DNA in the intermediate product.

The production strain was not detected in three batches of the intermediate concentrate used to formulate the additive, tested in triplicate [redacted].[3]

No recombinant DNA was detected in three batches of the intermediate concentrate, tested in triplicate by polymerase chain reaction (PCR), amplifying two fragments [redacted] corresponding to the recombinant mannanase genes present in the production strain.[4]

The final formulations of the additive have not been tested for the presence of cells and DNA from the strain. The intermediate product tested is a more concentrated product than the final additive.[5] Therefore, the results in the intermediate apply also for the additive.

3.2. Safety for the environment

The active substance is a protein and will be degraded/inactivated in the intestinal tract of the animals and therefore does not raise concern for the environment. However, in its previous assessment (EFSA FEEDAP Panel, 2017), the FEEDAP Panel could not conclude on the safety of the additive for the environment because uncertainty remained regarding the presence of the production strain and of its DNA. The applicant has now provided sufficient data supporting that the production strain and its recombinant DNA are not detected in the additive. Therefore, the additive under assessment manufactured by fermentation with *P. lentus* DSM 28088 does not raise any safety concern for the environment with regard to the genetic modification of the production strain.

3.3. Efficacy for pigs for fattening and minor porcine species

The additive is to be used in pigs for fattening and minor porcine species at 32,000 U/kg feed. In the previous assessment (EFSA FEEDAP Panel, 2017), a total of seven trials in pigs for fattening were evaluated, one short-term trial and six long-term trials. The results of the short-term trial showed no modifications of the metabolisable energy content of the diets by the use of the additive. The results of the long-term trials showed a significant and positive effect of the additive on the daily weight gain in only one trial. The applicant pooled the data of four of the studies and the statistical analysis indicated a significant improvement on the gain to feed ratio in the pigs receiving the additive as compared to the control group. However, the Panel did not consider the approach as valid, because the pooling should have been done with the six studies available which showed a similar design.

[3] Technical dossier FAD-2017-0070/Annex II.1.4.1.7.2 Conf.
[4] Technical dossier FAD-2017-0070/Annex II.2.2.2.7.2 Conf.
[5] The solid formulation, Hemicell® HT, ensures a minimum of 160 × 10^6 Units mannanase per kg and the liquid Hemicell® HT-L, ensures a minimum of 590 × 10^6 Units mannanase per L, the intermediate product tested contains 3,500 × 106 Units per kg.

Therefore, no conclusion could be drawn regarding the efficacy of the additive in pigs for fattening nor in minor porcine species for growing.

The applicant provided a new statistical analysis[6] pooling the data for the six long-term studies previously evaluated considering the data for the control groups and the group receiving 32,000 U/kg feed. For convenience, the design and results of the trials are presented in Table 2.

Table 2: Effect of Hemicell® HT on the performance of pigs for fattening

Trial	Breed Gender Duration	No. animals (animals/ replicate) Replicates/ treatment Initial body weight	Group (U/kg feed)	Daily feed intake (kg)	Final Body weight (kg)(1)	Daily weight gain (kg)	Feed to gain ratio(2)	Mortality (%)
1	Commercial breed ♂/♀ 92 days	288 (12) 12 23 kg	Control 32,000	1.52 1.54	87.1 89.3	0.699 0.722	2.17 2.14	3.4 2.0
2	Commercial breed ♂ castrated 106 days	200 (5) 20 27 kg	Control 32,000	2.61 2.64	129.0 132.0	0.967[a] 0.994[b]	2.70 2.65	2.0 2.0
3	Commercial breed ♂ castrated/♀ 104 days	208 (4-5) 21 26 kg	Control 32,000	2.69 2.72	129.1 129.5	0.993 0.995	2.71 2.73	1.9 1.9
4	Commercial breed ♂ castrated 84 days	252 (4-5) 26 31 kg	Control 32,000	2.61 2.60	119.1 119.9	1.05 1.06	2.48 2.46	2.3 0.8
5	Commercial breed ♂ castrated/♀ 82–84 days	40 (1) 20 23 kg	Control 32,000	2.63 2.60	103.5 103.6	0.975 0.975	2.70 2.67	0 0
6	Piétrain ♂castrated 83 days	50 (1) 25 26 kg	Control 32,000	2.63 2.64	113.7 115.8	1.06 1.09	2.47 2.42	0 0

(1): Values in trial 2 were calculated from the reported weight per pen.
(2): Values in trials 4 and 6 were calculated from the gain to feed reported in the studies.
[a,b]Values within one column and within one trial with different superscripts are significantly different ($p < 0.05$).

The data on daily feed intake, daily weight gain and feed to gain ratio from all trials were pooled and an analysis of variance (mixed model) was performed considering the effect of the treatment, study and their interaction and using the pen as experimental unit.

The results indicated no interactions between study and treatment in any of the parameters evaluated. No significant differences were found on the average daily feed intake (mean value 2.54 kg for the two groups) nor in the daily weight gain (mean value 0.977 g in control and 0.990 in the enzyme group). However, a significantly better feed to gain ratio was found in pigs receiving the additive at 32,000 U/kg feed compared to those from the control group (2.52 vs 2.55). Based on the results of this pooling analysis, the Panel concludes that the additive has a potential to be efficacious in pigs for fattening.

The mode of action of enzymes is well known and it is assumed to be the same among porcine species. Consequently, the Panel extrapolates the conclusion drawn in pigs for fattening to minor porcine species for growing and therefore concludes that the additive has a potential to be efficacious in minor porcine species for growing at 32,000 U/kg feed.

[6] Technical dossier FAD-2017-0070/Annex IV.3.18.

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[7] and Good Manufacturing Practice.

4. Conclusions

The production strain and its recombinant DNA were not detected in the intermediate product used to formulate the additive. The additive does not pose any environmental safety concern associated with the genetic modification of the production strain.

The additive has some potential to be efficacious in pigs for fattening and minor porcine species for growing when added to feed at 32,000 U/kg feed.

Documentation provided to EFSA

1) Supplementary information Hemicell® HT as a feed additive. December 2017. Submitted by Eli Lilly and Company Ltd.

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EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011. Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. EFSA Journal 2011;9(6):2193, 54 pp. https://doi.org/10.2903/j.efsa.2011.2193

Abbreviations

DSMZ	German Collection of Microorganisms and Cell Cultures
EC	enzyme commission
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
PCR	polymerase chain reaction

[7] 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.