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Safety and efficacy of Hemicell® HT (endo-1,4-β-D-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 (FEEDAP), Guido Rychen, Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Marta López Alonso, Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace, Pieter Wester, Paul Brantom, Noël Albert Dierick, Boet Glandorf, Lieve Herman, Sirpa Kärenlampi, Jaime Aguilera, Montserrat Anguita and Pier Sandro Cocconcelli

Abstract

Hemicell® HT is a feed additive with endo-1,4-β-D-mannanase as the main enzymatic activity that is available in solid (HT) and liquid (HT-L) forms. The production strain of the enzyme is a genetically modified strain of *Paenibacillus lentus.* The recipient strain is considered to be safe, the sequences introduced to obtain the production strain do not raise safety concerns. The absence of the production strain and of recombinant DNA in the product was not proven. The additive is safe for the target species at the corresponding recommended doses. The use of Hemicell® HT as a feed additive does not give rise to concerns for consumers. Hemicell® HT and Hemicell® HT-L are not irritant to the skin and eyes; Hemicell® HT is a skin sensitiser. No specific data were provided on the effects on the respiratory system; however, considering the nature of the active substance, the additive is considered a potential respiratory sensitiser. The active substance of the additive is a protein, and as such, it will be degraded/inactivated during passage through the digestive tract of the animals or in the environment. However, uncertainty remains on the absence of the production strain and of recombinant DNA in the product, consequently, the EFSA FEEDAP Panel could not conclude on the environmental safety of the product with regard to the genetically modified production strain. The additive has the potential to be efficacious in chickens for fattening at 32,000 U/kg feed and at 48,000 U/kg feed in turkeys for fattening and weaned piglets. These conclusions were extended to chickens reared for laying and turkeys reared for breeding and extrapolated to minor poultry species for fattening or reared for laying/breeding. The Panel could not conclude on the efficacy of the product in pigs for fattening or in minor porcine species.

Keywords: zootechnical additives, digestibility enhancers, safety, efficacy, beta-D-mannanase, chickens, laying hens

Requestor: European Commission

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Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Marta López Alonso, Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester.

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Summary

Following a request from the European Commission (EC), 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 Hemicell® HT (endo-1,4-β-D-mannanase) as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, weaned piglets, pigs for fattening and minor poultry and porcine species.

The additive contains endo-1,4-β-D-mannanase (mannanase) which is produced by a genetically modified strain of *Paenibacillus lentus*. The identity of the strain was confirmed as *P. lentus*. The recipient strain is considered to be safe, the sequences introduced to obtain the production strain do not raise safety concerns. The absence of the production strain and of recombinant DNA in the product was not proven.

Based on the tolerance trials evaluated, the FEEDAP Panel concluded that the additive is safe for chickens for fattening at 32,000 U/kg and for turkeys for fattening and weaned piglets at 48,000 U/kg feed. These conclusions were extended to chickens reared for laying and turkeys reared for breeding and to pigs for fattening at the corresponding doses. Based on the wide margin of safety shown in the major species, the conclusion was extrapolated to minor poultry species (for fattening or reared for laying/breeding) and growing minor porcine species.

Based on the results obtained in the genotoxicity studies and in a subchronic oral toxicity study, the Panel concluded that the use of Hemicell® HT as a feed additive is of no concern for consumers.

The FEEDAP Panel concluded that Hemicell® HT and Hemicell® HT-L are not irritant to the skin and eyes; Hemicell® HT is a skin sensitiser. No specific data were provided on the effects on the respiratory system; however, considering the nature of the active substance the additive was considered a potential respiratory sensitiser.

The active substance of the additive is a protein, and as such it will be degraded/inactivated during passage through the digestive tract of the animals or in the environment. However, since uncertainty remains on the absence of the production strain and on the possible presence of recombinant DNA in the product, the FEEDAP Panel could not conclude on the environmental safety of the product with regard to the genetically modified production strain.

Based on the results of the studies done in chickens and turkeys for fattening and weaned piglets, the FEEDAP Panel concluded that the additive has the potential to be efficacious in chickens for fattening at 32,000 U/kg feed and in turkeys for fattening and weaned piglets at 48,000 U/kg feed. These conclusions were extended/extrapolated to chickens reared for laying and turkeys reared for breeding at the corresponding doses. The conclusions reached in chickens for fattening were extrapolated to minor poultry species for fattening or reared for laying/breeding. The Panel could not conclude on the efficacy of the product in pigs for fattening or in minor porcine species.

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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 a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Eli Lilly and Company Ltd.[2] for authorisation of the product Hemicell® HT (endo-1,4-β-D-mannanase), when used as a feed 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 (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 17 April 2015.

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 Hemicell® HT (endo-1,4-β-D-mannanase), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The additive under assessment is not currently authorised for use in food and feed in the European Union (EU). It has not been previously assessed by EFSA as a feed additive.

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[3] in support of the authorisation request for the use of Hemicell® HT as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[4] 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.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Hemicell® HT 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, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c), Guidance on the

[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] Eli Lilly and Company Ltd. Eli Lilly Regional Operations Ges. m. b. H, Elanco Animal Health, Koelblgasse 8-10, A-1030, Vienna, Austria.
[3] FEED dossier reference: FAD-2014-0001.
[4] 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.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2014-0001-hemicell.pdf

assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012d), Technical Guidance: Microbial Studies (EFSA, 2008b), Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (2008c) 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

This assessment deals with the safety and efficacy of Hemicell® HT as a zootechnical additive (digestibility enhancer) 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.

3.1. Characterisation[6]

3.1.1. Characterisation of the active substance

The additive contains endo-1,4-β-D-mannanase (Enzyme Commission number: 3.2.1.78, mannanase) as the main enzyme activity. The applicant claims that there are no other side activities. This enzyme is produced by a genetically modified strain of *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.[7] The strain was shown not to be haemolytic,[8] not cytotoxic to VERO cells,[9] and is negative in the boar sperm mobility test.[10] Susceptibility to the relevant antibiotics listed in the Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012d) was shown for one of the intermediate strains during the genetic modification process.[11] The technical dossier contains detailed and sufficient information on the parental and recipient strains, including aspects on the safety of the strain lineage, the origin, modification and function of the different genetic elements modified in the production strain, the genetic modification process and the genetic and phenotypic traits modified.

3.1.2. Manufacturing process

The enzyme is obtained by submerged fermentation. After fermentation, the resulting product, liquid intermediate, is mixed with excipients for the preparation of the liquid formulation of the product or spray-dried and then mixed with excipients/carriers (see Section 3.1.3) to prepare the final solid formulation.

The applicant declared that no antimicrobial compounds, such as antibiotics, were used in the manufacturing process.

3.1.3. Characterisation of the additive

The additive is available in two forms, solid (Hemicell® HT) and liquid (Hemicell® HT-L).

The solid formulation, Hemicell® HT, ensures a minimum of 160×10^6 Units[12] mannanase per kg product. The study of the batch-to-batch variation in five batches showed a mean value of 241×10^6 U/kg ranging from 235×10^6 to 248×10^6 U/kg (coefficient of variation (CV) of 2.3%).[13] This formulation contains (w/w) linseed meal (58%), calcium carbonate (38%), mineral oil (1%; E905A paraffin) and dried fermentation product (3%, spray-dried with maltodextrin). Particle size distribution was measured in three lots by laser diffraction analysis[14] and showed a mean particle size between 239.8 and 260.7 μm, particles below 100 μm represented 13–18% (v/v), particles below 50 μm 5–9% (v/v) and particles below 10 μm represented 0.9–1.7% (v/v). The dusting potential was

[6] This section has been amended following the confidentiality claim made by the applicant.
[7] Technical dossier/Section II/Annex II.2.1.2.2.
[8] Technical dossier/Section II/Annex II.2.2.2.2.
[9] Technical dossier/Section II/Annex II.2.2.2.3.1.
[10] Technical dossier/Section II/Annex II.2.2.2.3.2.
[11] Technical dossier/Section II/Annex II.2.2.2.1.
[12] One unit is defined as the amount of mannanase enzyme which generates 0.72 mg of reducing sugars per minute from a mannan-containing substrate at pH 7.0 and 40°C.
[13] Technical dossier/Section II/Annex II.1.3.5.
[14] Technical dossier/Section II/II.1.5.2.

measured in three batches using the Stauber–Heubach method and ranged from 0.03 to 0.04 g/m^3.[15] The bulk density of the formulation ranges from 0.753 to 0.801 g/cm^3.[16]

The liquid formulation, Hemicell® HT-L, ensures a minimum of 590 × 10^6 Units mannanase per L product. The study of the batch-to-batch variation in five batches showed a mean value of 853 × 10^6 U/L ranging from 799 × 10^6 to 876 × 10^6 U/L (CV of 3.6%).[17] This formulation contains (w/w) sorbitol (48.6% (70% solution in water)), liquid fermentation product (20%), sodium chloride (8%), monosodium glutamate (4%), sodium acetate (0.7%), caramel colour (0.2%), potassium sorbate (0.1%), calcium chloride (0.1%) and added water (18.3%). The viscosity of this formulation ranges 8.2–10.4 Cp (measured at 25°C) and the surface tension 62.6–71.7 dynes/cm (measured at 23°C).[18]

Three batches of each final formulation were analysed for chemical and microbial contamination.[19] Chemical contamination included the analysis of lead (< 0.53 mg/kg in the solid and < 0.05 mg/kg in the liquid), cadmium (< 0.49 mg/kg in the solid and < 0.25 mg/kg in the liquid), mercury (< 0.1 mg/kg), antimony (< 0.5 mg/kg), bismuth (< 1.0 mg/kg), copper (< 11 mg/kg in the solid and < 0.5 mg/kg in the liquid), fluorine (< 10.2 mg/kg in the solid and < 5 mg/kg in the liquid), molybdenum (0.53 mg/kg in the solid and < 0.5 mg/kg in the liquid), silver (< 0.5 mg/kg), tin (< 2 mg/kg) and arsenic (0.50 mg/kg). The following mycotoxins were analysed, aflatoxins (B1, B2, G1 and G2, < 1.1 μg/kg), zearalenone (< 25 μg/kg), ochratoxin A (< 3.7 μg/kg), T2 toxin (< 25 μg/kg) fumonisins (< 1 mg/kg) and deoxynivalenol (0.5 mg/kg). Dioxins and dioxin-like PCBs were also analysed (WHO-PCDD/F-TEQ, < 1.5 ng/kg). Microbial contamination included the study of *Salmonella* spp. (absence in 25 g) in the two formulations and *E. coli* (not detected in 25 g), coliform bacteria (not detected) and aerobic plate counts (< 1,900 colony-forming units (CFU)/mL) in the liquid formulation.

The applicant investigated the presence of the production strain and its DNA in the intermediate product that is used to formulate the additive. Owing to the methodologies followed uncertainty remains on the presence of the production strain and its recombinant DNA in the additive.

Antimicrobial activity was found to be absent in the liquid culture supernatant and in the liquid intermediate used to formulate the additive, three batches each, following the method specified by the Joint FAO/WHO Expert Committee on Food Additives (FAO JECFA, 2006).[20]

3.1.4. Shelf life, stability and homogeneity

3.1.4.1. Shelf life

The shelf life of the solid formulation (three batches, mean enzyme activity 260 × 10^6 U/kg) was tested in samples stored in closed polypropylene bags at 5, 25, 30 and 40°C for 15, 24, 24 or 18 months, respectively.[21] After 15 months at 5°C, mean enzyme recovery was 87%. After 24 months storage at 25 or 30°C, the mean enzyme recoveries were 65% and 53%, and after 18 months at 40°C, recovery was 25%.

Shelf life of the liquid formulation (three batches, mean enzyme activity 830 × 10^6 U/L) was tested in samples stored in closed plastic bottles at 5, 25, 30 and 40°C up to 12 months.[22] After 12 months, samples stored at 5°C showed no modifications of the enzyme activity, samples stored at 25°C showed recoveries of the enzyme activity between 60% and 100%, and those stored at 30°C showed recoveries below 50%. After 9 months, samples kept at 40°C showed a complete loss of the enzyme activity.

3.1.4.2. Stability and homogeneity of the additive in premixtures and feedingstuffs

The stability of the solid formulation in complete vitamin–mineral premixtures for pigs was studied in three batches of the additive. One batch was added to a vitamin–mineral premixture for pigs (without choline chloride, supplemented at 59 × 10^6 U/kg) and the other two were added to a vitamin–mineral premixture for laying hens (with choline chloride, supplemented at 50 × 10^6 U/kg). Samples were stored in closed plastic bags at 25 or 30°C for 6 months or at 40°C for 2 months.[23] Enzyme recovery

[15] Technical dossier/Section II/Annex II.1.5.4.
[16] Technical dossier/Section II/Annex II.1.5.3.
[17] Technical dossier/Section II/Annex II.1.3.6.
[18] Technical dossier/Section II/Annex II.1.5.5.
[19] Technical dossier/Section II/Annex II.1.4.1.2.
[20] Technical dossier/Section II/Annex II.1.4.1.3 and Supplementary information November 2015/Annex II.1.4.1.6 and Supplementary information September 2016/Annex II.1.4.1.3.1.
[21] Technical dossier/Supplementary information September 2016/Annex II.4.1.1.2.
[22] Technical dossier/Section II/Annex II.4.1.2.
[23] Technical dossier/Section II/Annex II.4.1.5.

after 6 months storage at 25°C was 76% in the premixture for pigs and 34% in the layers' premixtures. The corresponding values for the samples stored at 30°C were 53% and 24%. After 2 months storage at 40°C, recovery was of 100% in the premixture for pigs and of 37.5% in the premixture for layers.

The stability of Hemicell® HT and Hemicell® HT-L was studied in two complete feed for poultry and in one feed for pigs in each formulation.[24] The additive was added to the mash feed at an intended dose of 32,000 or 48,000 U/kg feed. Mash feed was pelleted at two different temperatures 70–75°C and 80–85°C. Samples of mash and pelleted feed were stored at 25 and 30°C for 3 months or at 40°C for 2 months. Recoveries of enzyme activity after heat treatment were ~ 90% of the initial activity for the two formulations and the two temperatures tested. For the feed supplemented with the solid formulation, recovery values after 3 months in samples stored at 25°C was 100% in the mash and 94% for the pelleted samples. The corresponding values for samples kept at 30°C were 88% and 95%. After 2 months, the recovery in samples kept at 40°C were 83% for mash and pelleted feeds. For the feed supplemented with the liquid formulation, recovery values showed no enzyme activity losses in any of the conditions studied.

Ten subsamples of two of the premixtures and feeds used for the stability test were analysed in order to study the capacity of the additive to homogeneously distribute. The CV was 15.8% in the premixture for pigs and of 17% in the one for layers, in mash feed was of 12% and 7% for solid and liquid form, respectively, and in pelleted feed it was of 7% and 6%, respectively.

3.1.5. Conditions of use

The additive is to be used in chickens for fattening, chickens reared for laying, pigs for fattening and minor poultry or porcine species at a dose of 32,000 U/kg feed and in turkeys for fattening, reared for laying or for breeding and weaned piglets at 48,000 U/kg feed.

3.2. Safety

3.2.1. Safety aspects of the genetic modification[6]

The recipient organism is considered to be safe. Susceptibility to the antimicrobials listed in the Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012d) was demonstrated for an intermediate strain in the genetic modification process leading to the development of the production strain.[11] The introduced sequences raise no safety concern.

3.2.2. Safety for the target species

3.2.2.1. Safety for chickens for fattening

A total of 2,100 1-day-old male chickens (Ross 308) were penned in groups of 35 birds and allocated to one of five dietary treatments (representing 12 replicates per treatment).[25] Starter, grower and finisher basal diets based on wheat and soya protein were either not supplemented (control) or supplemented with the mannanase at 32,000 (1×), 48,000, 64,000 or 7,000,000 (218×) U/kg feed. The enzyme activity in the diets was confirmed with the exception of the diet with the highest supplementation level which showed recoveries similar to 65% of the intended dosage. Diets were offered to the birds for 42 days on *ad libitum* basis in pelleted form. Diets contained monensin sodium as a coccidiostat. Health status and mortality of the birds were monitored throughout the study. Feed intake and body weight were recorded and feed to gain ratio was calculated. Blood samples were collected from one bird per pen (12 birds per treatment) from the control group and animals from the two highest doses, and analysed for haematological[26] and biochemical parameters.[27] The same birds were subject to necropsy for gross pathology. An analysis of variance (ANOVA) was carried out with the data and group means were compared with Tukey test. The pen was the experimental unit.

Mortality was low and no differences were found between treatments (2.6%, 3.6%, 2.9%, 2.9% and 3.1% in the five groups). Mean total feed intake of the birds during the study was ~ 5.0 kg, mean final body weight was ~ 3.2 kg and mean feed to gain ratio was ~ 1.79 and were not affected by the dietary

[24] Technical dossier/Section II/Annex II.4.1.3 and II.4.1.4.
[25] Technical dossier/Section III/Annex III.1.1.1.
[26] Including: red blood cell counts, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin concentration, white blood cell counts including heterophil, lymphocyte, monocyte, eosinophil and basophil.
[27] Including aspartate aminotransferase, creatinine, calcium, phosphate, magnesium, urea, alkaline phosphatase, cholesterol, triglycerides and glucose.

treatments. No modifications were found on the haematological parameters studied. Biochemical parameters showed increases in the 64,000 dose compared to the control in the urea (1.10, 1.33, 1.27 mmol/L for control, 64,000 and 7,000,000 U/kg, respectively), cholesterol (3.12, 3.58 and 3.26 mmol/L, respectively) and calcium (2.90, 3.07 and 3.01 mmol/L, respectively), however, these differences were not present in the 7,000,000 group. Magnesium (1.23, 1.36, 1.42 mmol/L for 0, 64,000 and 7,000,000 U/kg) and phosphate (3.69, 4.09, 4.08 mmol/L) were higher in the supplemented groups as compared to the control. However, these modifications are considered to be of no concern. Therefore, the FEEDAP Panel considers that the additive is safe for chickens for fattening at the recommended dose.

3.2.2.2. Safety for turkeys for fattening

A total of 560 1-day-old male turkeys (Nicholas 85) were penned in groups of 10 birds and allocated to four dietary treatments (14 pens per treatment).[28] Starter and grower basal diets based on maize and soya bean meal were supplemented with the mannanase to provide 0, 48,000 (1×), 480,000 (10×) or 4,800,000 (100×) U/kg feed (confirmed by analysis). Diets were offered to the birds for 84 days on *ad libitum* basis and in pelleted form. The diets contained monensin sodium as a coccidiostat. Health status of the birds was monitored throughout the study. Feed intake, body weight and mortality were recorded and feed to gain ratio was calculated. Blood samples were collected from one bird per pen (14 per treatment) and analysed for haematological[29] and biochemical[30] parameters. An ANOVA was performed with the data (pen basis), and mean groups were compared to the control with Dunnett test.

Mortality was low and no differences were found between treatments (2.1%, 3.6%, 2.1% and 4.3%). Mean daily feed intake during the study (g/bird) was ~ 225 g, average daily weight gain was ~ 115 g and feed to gain ratio was ~ 1.92 and were not affected by the dietary treatments. Haematology and biochemical parameters results showed some statistical differences on the parameters measured. In the 10-fold dose compared to the control, there was a lower haemoglobin content (11.1, 10.7, 10.6 and 11.1 g/dL for control, 1×, 10× and 100×, respectively) and a higher triglyceride concentration (103, 103, 121 and 107 mg/dL), but these decreases were not found in the 100-fold dose. In the 100-fold dose, the uric acid concentration was higher compared to the control diet (5.1, 4.9, 5.7, 6.3 mg/dL for control, 1×, 10× and 100×, respectively). However, these changes are considered to be of no concern. Therefore, the FEEDAP Panel considers that the additive is safe for turkeys for fattening at the recommended dose.

3.2.2.3. Safety for weaned piglets

A total of 315 20-day-old weaned piglets (females and castrated males, commercial breed, initial body weight 6.4 kg) were allocated to pens in groups of five animals (sex separated) and allocated to three dietary treatments (representing 21 replicates per treatment).[31] Three basal diets (prestarter, starter and weaner) based on maize and soya bean meal were either not supplemented (control) or supplemented with the mannanase at 48,000 (1×) or 4,800,000 (100×) U/kg feed. The analysis of the 100-fold diet showed recoveries close to 70% of the intended dosage. Feed was offered to the piglets for 42 days on *ad libitum* basis in pelleted form. Health condition of the animals and mortality were monitored throughout the study. Piglets were weighed and feed intake was measured on days 14, 28 and 42 and feed to gain was calculated. On day 42, one pig per pen was randomly selected and blood was collected and analysed for haematological[32] and biochemical[33] parameters. An ANOVA was performed with the data (pen basis) and group means were compared with the least significant difference (LSD) test.

One piglet from the onefold diet and one from the 100-fold groups died. Three piglets were removed from the study due to lameness; the three belonged to the 100-fold diet. Compared to the

[28] Technical dossier/Section III/Annex III.1.1.2.

[29] Including: haematocrit, haemoglobin, white blood cell counts (including differential) and thrombocytes.

[30] Including: albumin, globulin (and ratio), chloride, cholesterol, glucose, potassium, sodium, total protein, triglycerides, uric acid, gamma-glutamyl transpeptidase, alanine aminotransferase, aspartate transaminase, blood urea nitrogen, creatinine and total bilirubin.

[31] Technical dossier/Section III/Annex III.1.1.3 and Supplementary information November 2015/Annex III.1.1.3_amendment.

[32] Including: red blood cells count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red blood cell distribution width, white blood cells (including differential), platelet concentration and mean platelet volume.

[33] Including: creatinine, blood urea nitrogen, total bilirubin, sodium, potassium, chloride, total protein, albumin, globulin, calcium, phosphorus, magnesium, cholesterol, glucose, alkaline phosphatase, aspartase aminotransferase, creatine kinase, gamma-glutamyl transpeptidase, glutamate dehydrogenase, beta-hydroxytutyrate, non-esterified fatty acid, carbon dioxide, ion-gap.

control diet, the diets containing the mannanase had a significantly higher final body weight (24.4, 26.3 and 26.2 kg for control, 1× and 100×, respectively) and a significantly higher daily feed intake (681, 720 and 718 g/pig, respectively). The feed to gain ratio was significantly improved with the diets containing the mannanase as compared to the control (1.58, 1.51 and 1.51 for control, 1× and 100×, respectively).[34] Haematological parameters showed a decrease in the platelet concentration in the supplemented diets (570.6, 417.1 and 441.8 K/μL for control, 1× and 100×, respectively). Compared to the control diet, the onefold diet showed lower blood urea nitrogen values (10.1, 7.9 and 8.9 mg/dL for control 1× and 100×, respectively) and cholesterol (94.6, 85.3 and 86.7 mg/dL) and higher glucose concentrations (105, 114 and 108 mg/dL). In the 100-fold diet, alkaline phosphatase (255, 242 and 216 U/L) and gamma-glutamyl transpeptidase (32.6, 30.1 and 25.0 U/L) were lower compared to control. A lower globulin (1.90 vs 1.60 and 1.61 g/dL) and higher albumin (2.86 vs 3.29 and 3.39 g/dL) were found in the supplemented groups compared to control. However, these changes are considered to be of no concern. Therefore, the FEEDAP Panel considers that the additive is safe for the weaned piglets at the recommended dose.

3.2.2.4. Safety for pigs for fattening

No specific study was provided to demonstrate the tolerance for pigs for fattening. The FEEDAP Panel considers that the conclusions reached for piglets can be extended to pigs for fattening.

3.2.2.5. Conclusions on safety for the target species

Based on the studies provided in chickens and turkeys for fattening and in piglets, the FEEDAP Panel concludes that the additive is safe at 32,000 U/kg feed for chickens for fattening and at 48,000 U/kg feed in turkeys for fattening and weaned piglets. These conclusions can be extended to chickens reared for laying at 32,000 U/kg feed and to turkeys reared for breeding and pigs for fattening at 48,000 U/kg feed. Considering the wide margin of safety shown in these studies, the conclusions are extrapolated to minor poultry species (for fattening or raised for laying/breeding) and to growing minor porcine species at 48,000 U/kg feed.

3.2.3. Safety for the consumer

3.2.3.1. Genotoxicity

Bacterial reverse mutation assay

The fermentation product that is used to prepare the additive was tested to evaluate its mutagenic potential in *Salmonella* Typhimurium strains TA98, TA100, TA1535 and TA1537 and in *E. coli* strain WP2 *uvr*A in the presence and absence of an exogenous metabolic activation system (Aroclor 1,254-induced rat liver S9) up to a maximum concentration of 5,000 μg per plate, in compliance with the Organisation for Economic Co-operation and Development (OECD) Guideline 471.[35] Water was used as the vehicle. Two independent experiments were performed. Neither precipitate nor toxicity was observed. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of metabolic activation, while the positive controls performed as expected.

In vitro mammalian cell micronucleus test

The fermentation product that is used to prepare the additive was tested to evaluate the potential to induce micronuclei in Chinese hamster ovary (CHO) cells in both the absence and presence of an exogenous metabolic activation system (Aroclor 1254-induced rat liver S9) in compliance with the OECD Guideline 487.[35] Water was used as the vehicle. Based on the results of a preliminary toxicity assay, the concentrations selected for testing ranged from 0.2 to 2,000 μg/mL. Two treatment schedules were applied: 4-h treatment followed by 20-h recovery in the presence of cytochalasine-B, with and without metabolic activation; 24-h treatment in the presence of cytochalasine-B, only without metabolic activation. Cytotoxicity (58% cytokinesis-blocked proliferation index) was observed only at the top concentration only in the 24-h exposure group without metabolic activation.

No significant or dose-dependent increases in micronuclei induction were observed in any group treated with the test item, with or without S9 ($p > 0.05$; Fisher's Exact and Cochran–Armitage tests), while the positive controls induced significant increases in the percentage of micronucleated cells.

[34] Feed to gain ratio was calculated from the gain to feed ratio reported in the study report.
[35] Technical dossier/Supplementary information September 2016/Annex III.2.2.2.1.1.

3.2.3.2. Subchronic oral toxicity study

The systemic toxic potential of the fermentation product used to prepare the additive to rats by oral administration was assessed over a period of 13 weeks, in compliance with OECD Guideline 408.[36] The test was preceded by a dose-range finding study in which oral doses up to 1,000 mg/kg body weight (bw) and day were well tolerated. Four groups of 10 Crl:CD® SD rats of each sex received the test material by gavage (10 mL/kg bw per day) for 91 days at doses of 0, 250, 500 or 1,000 mg/kg bw per day. The control group received the vehicle (distilled water). During the study, clinical condition, body weight and food consumption were recorded. Detailed clinical observations were conducted weekly and functional evaluations and ophthalmic examinations were conducted at the beginning and at the end of the study. Blood samples were taken at the end of the study from all animals for haematology[37] and blood chemistry measurements.[38] Urine samples were collected for analysis. All animals were subject to a necropsy during which organ weights were recorded,[39] gross pathology observations were made and tissues samples preserved for histological examination. Slides were prepared from tissues collected from control and high-dose groups and were examined microscopically for histopathological changes.

There were no effects of treatment on mortality, clinical observations, ophthalmoscopy or functional observations. During the study, the food intake and body weight of all groups was similar. High-dose females had significantly lower counts of reticulocytes and neutrophils compared with controls, but a similar effect was not seen in males. High-dose males had a significantly shorter mean prothrombin time but this difference was not reproduced in females. Serum urea nitrogen was slightly higher than controls in high-dose females. There were no differences in organ weights or histopathology which could be attributed to treatment. This study did not identify any effects of treatment which would be of concern.

A further subchronic oral toxicity study performed in compliance with the OECD Guideline 408 was provided. The test item used in that study was derived from the parental strain of the current production strain. The results showed no treatment-related effect. The Panel considers this study as supporting evidence of the safety of the product.

3.2.3.3. Conclusions on the safety for the consumer

The results obtained with the fermentation product used to prepare the additive in the genotoxicity studies and in the subchronic oral toxicity study do not indicate any reason for concern for consumer safety arising from the use of the product as a feed additive.

3.2.4. Safety for the user

3.2.4.1. Effects on the respiratory system

No specific studies were submitted. Owing to the proteinaceous nature of the active substance, the additive is considered a potential respiratory sensitiser. The solid formulation has particles below 10 μm but the dusting potential is low, consequently, the potential exposure is expected to be limited.

3.2.4.2. Effects on the skin and eyes

The acute dermal irritant potential of Hemicell® HT and Hemicell® HT-L was investigated in rabbits in compliance with the OECD Guideline 404.[40] No dermal irritation was observed in any animal at any time point during the study. The test materials were classified as not irritant to the skin.

The eye irritancy potential of Hemicell® HT and Hemicell® HT-L was investigated in accordance with the OECD Guideline 405.[41] No effects were seen 24 h post-dose and later. The test materials were classified as not irritant to the eyes.

[36] Technical dossier/Supplementary information November 2015/Annex III.2.2.5.
[37] Including: haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean crorpuscular haemoglobin content, red blood cells, leucocytes (including differential), platelets, prothrombin time, fibrinogen, thromboplastin partial time.
[38] Including: sodium, potassium chloride, calcium, phosphorus, alkaline phosphatase, total bilirubin, alanine transaminase, aspartate transaminase, urea nitrogen, creatinine, total protein, albumin, globulin, triglyceride, cholesterol and glucose.
[39] Including the adrenals, brain, heart, kidney, liver, pituitary gland, prostate, spleen, thymus, thyroid/parathyroid, uterus, epididymis, ovaries, testis.
[40] Technical dossier/Section III/Annex III.3.1.2.1 and Annex III.3.1.2.4.
[41] Technical dossier/Section III/Annex III.3.1.2.2 and Annex III.3.1.2.5.

The potential of Hemicell® HT and Hemicell® HT-L to cause or elicit skin sensitisation reactions (allergic contact dermatitis) was assessed via the murine local lymph node assay (MLLNA), in compliance with the OECD Guideline 429.[42] Hemicell® HT exhibited skin sensitisation potential but not Hemicell® HT-L.

3.2.4.3. Conclusions on safety for the user

Hemicell® HT and Hemicell® HT-L are not irritant to the skin and eyes; Hemicell® HT is a skin sensitiser. The additive is considered a potential respiratory sensitiser.

3.2.5. Safety for the environment

The active substance of the additive is a protein, and as such it will be degraded/inactivated during passage through the digestive tract of the animals or in the environment. However, uncertainty remains on the absence of the production strain and on the absence of recombinant DNA in the product. If those were present, the product should be subjected to environmental risk assessment according to the Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011). Therefore, the EFSA FEEDAP Panel cannot conclude on the environmental safety of the product.

3.3. Efficacy

3.3.1. Efficacy for chickens for fattening

A total of four long-term trials were evaluated. The first three studies were conducted in the same place and followed a similar study design. The fourth, conducted in another trial site, is the tolerance trial presented in Section 3.2.2.1. The details of the design of the four studies are shown in Table 1 and the results of the trials in Table 2. In the four trials, 1-day-old male birds were used and were under study for 42 days. In all trials, the basal diets (starter, grower and finisher) were either not supplemented (control) or supplemented with the mannanase contained in the additive at different inclusion levels (solid form in trials 1–3 and liquid intermediate in trial 4). The enzyme activities in the feed were analysed. The recommended dosage (32,000 U/kg feed) was included in trials 1, 3 and 4. In trial 1, the study design considered two different energy contents of the diets, high (H: 12.8 MJ metabolisable energy (ME)/kg feed) or low (L: 12.5 MJ ME/kg feed). General health status and mortality were monitored throughout the studies. Feed intake and body weight were measured and feed to gain ratio was calculated. In each study, an ANOVA was performed with the data (pen basis) and group means were compared using the Student–Newman–Keuls (trial 1) or Tukey test (trials 2, 3 and 4). Differences were considered statistically significant at a level of at least $p < 0.05$.

The mortality was within the normal levels and no differences between the groups were identified. The supplementation with mannanase improved the feed to gain ratio in three trials (1, 2 and 3) from the nominal dose of 16,000 U/kg feed in trials 1 and 3 and from 22,400 U/kg feed in trial 2. The supplementation of mannanase increased the body weight gain in one trial (trial 3) from the nominal dose of 16,000 U/kg feed. The analysis of the diets showed that in general the enzyme activity in the supplemented diets supplemented were higher than the intended. In the groups with the lowest enzyme activity showing significant effects, the analysed enzyme activity was 25,800, 31,000 and 14,600 U/kg feed. Therefore, the Panel considers that the additive has a potential to be efficacious in chickens for fattening at the recommended dose (32,000 U/kg feed).

[42] Technical dossier/Section III/Annex III.3.1.2.3 and Annex III.3.1.2.6.

Table 1: Trial design and dosages of the efficacy trials performed in chickens for fattening

Trial	Breed Gender Duration	Total no. of animals (animals/replicate) Replicates/treatment	Basal diets composition (feed form)	Enzyme activity (U/kg feed)	
				Intended	Analysed
1[(a)]	Cobb × Cobb Males 42 days	2,800 (50) 8	Maize, soya bean meal (pelleted)	0 (H) 32,000 (H) 0 (L) 16,000 (L) 32,000 (L) 48,000 (L) 64,000 (L)	2,433 43,533 4,066 29,866 38,533 56,100 77,800
2[(b)]	Cobb × Cobb Males 42 days	1,050 (50) 7	Maize, soya bean meal (pelleted)	0 22,400 48,000	3,700 34,666 60,133
3[(c)]	Cobb × Cobb Males 42 days	1,600 (50) 8	Maize, soya bean meal (pelleted)	0 16,000 32,000 48,000	8,766 23,433 39,333 52,033
4[(d)]	Ross 308 Males 42 days	2,100 (35) 12	Wheat, soya protein (pelleted)	0 32,000 48,000 64,000 7,000,000	24,333 69,466 71,900 82,700 6,487,200

(a): Technical dossier/Section IV/Annex IV.3.1.
(b): Technical dossier/Section IV/Annex IV.3.2 and Supplementary information November 2015.
(c): Technical dossier/Section IV/Annex IV.3.3 and Supplementary information November 2015/Annex IV.3.3.
(d): Technical dossier/Section IV/Annex III.1.1.

Table 2: Effects of Hemicell® HT on the performance of chickens for fattening

Trial	Treatment	Feed intake[(1)]	Body weight gain (g)	Feed to gain ratio	Mortality (%)
1	0 (H)	188	2,183[a]	1.82[a]	6.5
	32,000 (H)	193	2,297[a]	1.78[a]	7.7
	0 (L)	190	2,030[c]	1.93[b]	5.0
	16,000 (L)	186	2,130[bc]	1.82[a]	5.5
	32,000 (L)	188	2,158[abc]	1.81[a]	6.5
	48,000 (L)	185	2,160[abc]	1.82[a]	8.5
	64,000 (L)	183	2,145[bc]	1.80[a]	8.0
2	0	170	1,760	1.99[a]	7.7
	22,400	170	1,800	1.93[b]	7.1
	48,000	168	1,799	1.91[b]	6.9
3	0	192[b]	2,135[b]	1.85[a]	5.5
	16,000	202[a]	2,311[a]	1.75[b]	2.7
	32,000	199[ab]	2,294[a]	1.75[b]	3.0
	48,000	194[ab]	2,283[a]	1.74[b]	4.5
4	0	5,686	3,167	1.79	2.6
	32,000	5,722	3,210	1.78	3.6
	48,000	5,830	3,232	1.80	2.9
	64,000	5,652	3,180	1.78	2.9
	7,000,000	5,673	3,190	1.78	3.1

(1): Values in trial 1, 2 and 3 are feed intake per pen in kilogram, in trial 4 are total feed intake per bird in gram.
a,b,c: Values within one column and within one trial with different superscripts are significantly different ($p < 0.05$).

3.3.2. Efficacy for turkeys for fattening

A total of four long-term trials were evaluated; trial number 3 is the tolerance trial presented in Section 3.2.2.2. The details of the design of the four studies are shown in Table 3 and the results of the trials in Table 4. In all trials, 1-day-old birds were used and were under study for 84 days. The basal diets (different number of diets according to the nutritional requirements of birds) were either not supplemented (control) or supplemented with the additive (liquid form in trial 1 and solid in the other trials) at different dosages. The recommended dose of 48,000 U/kg feed was considered in all trials. The enzyme activities were analysed in all trials. General health status and mortality were monitored throughout the studies. Feed intake and body weight were measured and feed to gain ratio was calculated. In each study, an ANOVA was performed with the data (pen basis) and group means were compared using the Tukey test (trials 1 and 4) or Dunnett (trials 2 and 3). Differences were considered statistically significant at a level of at least $p < 0.05$.

Table 3: Trial design and dosages of the efficacy trials performed in turkeys for fattening

Trial	Breed Gender Duration	Total no. of animals (animals/replicate) Replicates/treatment	Basal diets composition (feed form)	Enzyme activity (U/kg feed)	
				Intended	Analysed
1[(a)]	BUT Big 6 females 84 days	540 (12) 15	Maize, soya bean meal (pelleted)	0 32,000 48,000	3,850 34,875 49,950
2[(b)]	Nicholas 85 females 84 days	420 (15) 14	Maize, soya bean meal (pelleted)	0 48,000	6,000 62,250
3[(c)]	Nicholas 85 Males 84 days	560 (10) 14	Maize, soya bean meal (pelleted)	0 48,000 480,000 4,800,000	7,875 48,475 417,175 3,931,600
4[(d)]	BUT Big 6 females 84 days	600 (10) 20	Wheat, barley, soya bean meal (pelleted)	0 32,000 48,000	402 38,550 58,125

(a): Technical dossier/Section IV/Annex IV.3.4.
(b): Technical dossier/Section IV/Annex IV.3.5.
(c): Technical dossier/Section IV/Annex III.1.1.2.
(d): Technical dossier/Supplementary information November 2015/Annex IV.3.10.

Mortality was low and no significant differences between groups were identified. The supplementation of the diets with the mannanase improved the feed to gain ratio in two trials (trials 1 and 3) at 48,000 U/kg feed and in another trial (trial 4) improved the body weight gain and feed to gain ratio from the dose of 32,000 U/kg feed. Therefore, the Panel considers that the additive has a potential to be efficacious for turkeys for fattening at the recommended dose (48,000 U/kg feed).

Table 4: Effects of Hemicell® HT on the performance of turkeys for fattening

Trial	Treatment	Daily feed intake (g)	Body weight gain (g)[1]	Feed to gain ratio	Mortality (%)
1	0	210	8,258	2.15[a]	1.7
	32,000	210	8,417	2.11[a]	1.1
	48,000	206	8,342	2.09[b]	1.7
2	0	199[a]	95	2.09[a]	2.2
	48,000	196[b]	95	2.05[b]	3.1
3	0	224	117	1.91	2.1
	48,000	222	115	1.92	3.6
	480,000	224	116	1.92	2.1
	4,800,000	224	115	1.94	4.1
4	0	204	95[b]	2.16[a]	0
	32,000	202	98[a]	2.07[b]	1.0
	48,000	206	99[a]	2.07[b]	1.0

(1): Values in trial 1 are total body weight gain and in trials 2–4 are individual body weight gain per day.
a,b,c: Values within one column and within one trial with different superscripts are significantly different ($p < 0.05$).

3.3.3. Efficacy for weaned piglets

One short-term trial and four long-term trials were submitted by the applicant.

3.3.3.1. Short-term trial

A total of 72 43-day-old castrated male piglets (body weight 10.8 kg, (Large White × Landrace) × Duroc) were individually penned and distributed to three dietary treatments.[43] A basal diet based on maize, soya bean meal and maize dried distillers grain solubles was either not supplemented (control) or supplemented with the mannanase to provide 32,000 or 48,000 U/kg feed (analysed values: 9,700, 52,100 and 75,700 U/kg). Piglets were fed the experimental diets on *ad libitum* basis for 7 days. After this adaptation period, a total of 48 piglets were transferred to metabolic cages, representing 16 replicates per treatment. The piglets were kept in the metabolic cages for 7 days and fed the corresponding diets (amount equal to 9% of the metabolic body weight in two equal daily portions). Body weight of the piglets was measured on days 0, 7 and 14, feed intake was measured. Total collection of faeces and urine was carried out on the last four days of study. Total weight, dry matter content, total nitrogen and energy content were determined in feed, faeces and urine. The ME content of the diets was determined. An ANOVA was performed with the data and group means were compared with Tukey test. Differences were considered statistically significant at a level of at least $p < 0.05$.

No animals died or were removed from the study. The content of ME of the diets was significantly improved by Hemicell® HT addition only at the nominal dose of 32,000 U/kg feed (analytical value 42,000 U/kg feed) from 13.6 to 14.2 MJ/kg feed.

3.3.3.2. Long-term trials

A total of four long-term trials were evaluated. Trial number 2 is the tolerance trial presented in Section 3.2.2.3. The details of the design of the four studies are shown in Table 5 and the results of the trials in Table 6. In all trials, the basal diets (different number of diets according to the nutritional requirements of the pigs) were either not supplemented (control) or supplemented with the additive (liquid form in trial 1 and solid in the other trials) at different dosages. The recommended dose of 48,000 U/kg feed was considered in all trials. The enzyme activities were analysed in all trials. General health status and mortality were monitored throughout the studies. Feed intake and body weight were measured and feed to gain ratio or gain to feed were calculated. In each study, an ANOVA was performed with the data (pen basis) and group means were compared using the Tukey test (trials 1 and 2) or Dunnett (trials 3 and 4). Differences were considered statistically significant at a level of at least $p < 0.05$.

The results of the single studies showed a significant effect of the treatment in only one trial (trial 2). The data on daily feed intake, daily weight gain and the gain to feed ratio from the four studies

[43] Technical dossier/Section IV/Annex IV.2.1 and Supplementary information November 2015/Annex IV.2.1.

were pooled and statistically analysed.[44] The treatments included were the control and the recommended dose (48,000 U/kg feed). The statistical model considered the effect of the treatment and the study as main effects as well as their interaction. The results showed no significance of the interaction in any of the parameters evaluated. Hemicell® HT at 48,000 U/kg feed significantly improved the gain to feed ratio as compared to the control (1.65 vs 1.61, $p = 0.03$).[45] No other significant effects were found.

The FEEDAP Panel concludes that the additive has a potential to be efficacious in weaned piglets at the recommended dose (48,000 U/kg feed).

Table 5: Trial design and dosages of the efficacy trials performed in weaned piglets

Trial	Breed Gender(e) Duration	Total no. of animals (animals/replicate) Replicates/treatment	Basal diets composition (feed form)	Enzyme activity (U/kg feed)	
				Intended	Analysed
1(a)	Large White × Landrace ♂ 42 days	96 (4) 8	Wheat, barley, soya bean (pelleted)	0 32,000 48,000	16,100 49,600 65,400
2(b)	Commercial breed ♂ castrated/♀ 42 days	315 (5) 21	Maize, soya bean meal (pelleted)	0 48,000 4,800,000	8,000 36,000 3,460,000
3(c)	Commercial breed ♂ castrated 42 days	390 (10) 13	Maize, soya bean meal (pelleted/mash)	0 32,000 48,000	10,800 44,500 56,600
4(d)	Commercial breed ♂/♀ 42 days	120 (4) 10	Maize, barley, soya bean meal (pelleted)	0 32,000 48,000	11,500 58,150 66,600

(a): Technical dossier/Section IV/Annex IV.3.6.
(b): Technical dossier/Section IV/Annex III.1.1.3 and Supplementary information November 2015/Annex III.1.1.3.
(c): Technical dossier/Supplementary information November 2015/Annex IV.3.11.
(d): Technical dossier/Supplementary information November 2015/Annex IV.3.12.
(e): In trial 2 – sex separated; in trial 4 – randomly distributed to treatments.

Table 6: Effects of Hemicell® HT on the performance of weaned piglets

Trial	Treatment	Feed intake (kg)(1)	Initial body weight (kg)	Final body weight gain (kg)	Feed to gain ratio(2)	Mortality (%)
1	0	53.9	9.7	36.4	2.02	0
	32,000	54.2	9.8	36.6	2.02	0
	48,000	55.1	9.6	37.0	2.01	0
2	0	0.681[b]	6.4	24.4[b]	1.58[b]	0
	48,000	0.720[a]	6.4	26.3[a]	1.51[a]	1
	4,800,000	0.718[a]	6.4	26.2[a]	1.52[ab]	4
3	0	0.805	7.7	31.2	1.43	3
	32,000	0.816	7.7	31.5	1.44	1.5
	48,000	0.788	7.7	30.8	1.43	3
4	0	0.828	6.7	25.0	1.89	0
	32,000	0.878	6.7	26.6	1.84	0
	48,000	0.819	6.7	25.4	1.84	3

(1): Values in trial 1 are total feed intake per pig and in trials 2–4 are individual daily feed intake.
(2): Values in trials 2–4 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 ($p < 0.05$).

[44] Technical dossier/Supplementary information November 2015/Annex IV.3.13.
[45] Values calculated from the gain to feed reported in the study.

3.3.4. Efficacy for pigs for fattening

One short-term trial and six long-term trials were submitted by the applicant.

3.3.4.1. Short-term trial

A total of 72 castrated male pigs (body weight 45 kg, (Large White × Landrace) × Duroc)) were penned in groups of six animals and distributed to three dietary treatments. A basal diet based on maize, wheat middlings, soybean hulls and soya bean meal was either not supplemented (control) or supplemented with Hemicell® HT to provide 0, 32,000 or 48,000 U/kg feed (confirmed by analysis: 2,205, 40,022 and 54,300 U/kg). Pigs were fed the experimental diets on *ad libitum* basis for 14 days. After this adaptation period, a total of 60 pigs were randomly selected and transferred to metabolic cages, representing 20 replicates per treatment. The pigs were kept in the metabolic cages for 7 days and fed the corresponding diets (amount equal to 9% of the metabolic body weight in two equal daily portions). Body weight of the pigs was measured on days 0, 14 and 21 and feed intake was measured for these 14 days. Total collection of faeces and urine was carried out on the last four days of study. Total weight, dry matter content, total nitrogen and energy content were determined in feed, faeces and urine. The ME content of the diets was determined. An ANOVA was performed with the data and group means were compared with a LSD test.

No animals died or were removed from the trial. The ME content in the diets was not affected by the treatment (mean value 13.8 MJ/kg feed, $p = 0.78$).

3.3.4.2. Long-term trials

A total of six long-term trials were evaluated. The details of the design of the four studies are shown in Table 7 and the results of the trials in Table 8. In all trials, the basal diets (different number of diets according to the nutritional requirements of the pigs) were either not supplemented (control) or supplemented with the additive (solid form) at different dosages. The recommended dose of 32,000 U/kg feed was considered in all trials. The enzyme activities were analysed in all trials. General health status and mortality were monitored throughout the studies. Feed intake and body weight were measured (at different time points, see Table 7) and feed to gain ratio or gain to feed were calculated. In each study, an ANOVA was performed with the data (pen basis) and group means were compared using the Tukey test (trials 1, 2 and 6), LSD (trial 3) or Dunett (trials 4 and 5). Differences were considered statistically significant at a level of at least $p < 0.05$.

The results of the single studies showed a significant effect of the treatment in only one trial (trial 2, daily weight gain).

The data on daily feed intake, daily weight gain and the gain to feed ratio from four studies (2, 4, 5 and 6) were pooled by the applicant and statistically analysed.[46] The analysis was done on the average weight gain, daily feed intake and gain to feed ratio measured on days 82–84 (days under study). The data considered included the control and the recommended dose (32,000 U/kg feed). The statistical model considered the effect of the treatment and the study as main effects as well as their interaction. The results showed no significance of the interaction in any of the parameters evaluated. Hemicell® HT at 32,000 U/kg feed significantly improved the gain to feed ratio as compared to the control (2.52 vs 2.48, $p = 0.009$).[45] No other significant effects were found.

The applicant was requested to pool the data from the six studies available. This request was not followed. The applicant explained that trials 1 and 3 were not considered because the parameters were not measured on day 82 of study; closest day in trial 1 was day 92 and in trial 3 day 77. The FEEDAP Panel considers this exclusion as a weak point in the statistical approach. The data from the six trials could be pooled and consideration of the different duration be done in the statistical analysis. Therefore, the Panel is not in the position to accept such approach and considers that no conclusion can be drawn from the data provided by the applicant.

[46] Technical dossier/Supplementary information November 2015/Annex IV.3.17 and Supplementary information September 2016.

Table 7: Trial design and dosages of the efficacy trials performed in pigs for fattening

Trial	Breed Gender[g] Duration (day controls)	Total no. of animals (animals/replicate) Replicates/treatment	Basal diets composition (feed form)	Enzyme activity (U/kg feed)	
				Intended	Analysed
1[a]	Commercial breed ♂/♀ 92 days (0, 42, 92)	432 (12) 12	Barley, soya bean and sunflower meal (pelleted)	0 32,000 48,000	8,800 34,600 46,200
2[b]	Commercial breed ♂ castrated 106 days (0, 28, 57, 82, 106)	300 (5) 20	Maize, soya bean meal (pelleted)	0 32,000 48,000	8,800 35,000 46,000
3[c]	Commercial breed ♂ castrated/♀ 104 days (0, 28, 56, 77, 104)[h]	309 (4–5) 21	Maize, soya bean meal (mash)	0 32,000 48,000	4,200 37,400 49,300
4[d]	Commercial breed ♂ castrated 84 days (0, 21, 42, 70, 84)	380 (4–5) 26	Maize, soya bean meal (mash)	0 32,000 48,000	8,500 46,800 77,000
5[e]	Commercial breed ♂ castrated/females 82–84 days (0, 28, 55, 77, 84)	60 (1) 20	Wheat, barley, maize, soya bean meal (pelleted/mash)	0 32,000 48,000	7,200 45,000 49,600
6[f]	Piétrain ♂ 83 days (0, 37, 64, 83)	50 (1) 25	Wheat, barley, rye, soya bean meal (pelleted)	0 32,000	12,700 55,000

(a): Technical dossier/Section IV/Annex IV.3.7 and Supplementary information November 2015/Annex IV.3.7.
(b): Technical dossier/Section IV/Annex IV.3.8 and Supplementary information November 2015/Annex IV.3.8.
(c): Technical dossier/Section IV/Annex IV.3.9.
(d): Technical dossier/Supplementary information November 2015/Annex IV.3.14.
(e): Technical dossier/Supplementary information November 2015/Annex IV.3.15.
(f): Technical dossier/Supplementary information November 2015/Annex IV.3.16.
(g): In trial 1 and 3, animals were sex separated.
(h): According to Supplementary information September 2016, measurements were done on days 0, 28, 56 and 98.

Table 8: Effect of Hemicell® HT on the performance of pigs for fattening

Trial	Treatment	Daily feed intake (kg)	Body weight (kg)(1)		Daily weight gain (kg)	Feed to gain(2)	Mortality (%)
			Initial	Final			
1	0	1.52	22.9	87.1	0.699	2.17	3.4
	32,000	1.54	22.9	89.3	0.722	2.14	2.0
	48,000	1.54	22.9	88.9	0.719	2.14	0.7
2	0	2.61	27.5	129.0	0.967[a]	2.70	2.0
	32,000	2.64	27.4	132.0	0.994[b]	2.65	2.0
	48,000	2.63	27.5	131.9	0.989[ab]	2.66	2.0
3	0	2.69	25.7	129.1	0.993	2.71	1.9
	32,000	2.72	25.7	129.5	0.995	2.73	1.9
	48,000	2.69	25.8	130.8	1.00	2.68	4.9
4	0	2.61	31.1	119.1	1.05	2.48	2.3
	32,000	2.60	31.4	119.9	1.06	2.46	0.8
	48,000	2.64	31.9	120.9	1.06	2.48	0.8
5	0	2.63	22.7	103.5	0.975	2.70	0
	32,000	2.60	22.7	103.6	0.975	2.67	0
	48,000	2.61	22.7	102.4	0.961	2.72	0
6	0	2.63	25.6	113.7	1.06	2.47	0
	32,000	2.64	25.5	115.8	1.09	2.42	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 ($p < 0.05$).

3.3.5. Conclusions on the efficacy

Based on the results of the efficacy trials evaluated, the Panel concludes that the additive has a potential to be efficacious in chickens for fattening at 32,000 U/kg feed and at 48,000 U/kg feed in turkeys for fattening and weaned piglets. The Panel considers that these conclusions can be extended to chickens reared for laying and turkeys reared for breeding at the corresponding doses. The conclusions reached in chickens for fattening can be extrapolated to minor poultry species for fattening or reared for laying/breeding.

The Panel cannot conclude on the efficacy of the product in pigs for fattening or in growing minor porcine species.

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[47] and Good Manufacturing Practice.

4. Conclusions

The recipient strain is considered to be safe, the sequences introduced to obtain the production strain do not raise safety concerns and there are no antibiotic resistance genes from the genetic modification in the production strain. The absence of the production strain and of recombinant DNA in the product has not been proven.

The additive is safe for chickens for fattening at 32,000 U/kg feed and in turkeys for fattening and weaned piglets at 48,000 U/kg feed. These conclusions can be extended to chickens reared for laying and turkeys reared for breeding and to pigs for fattening at the corresponding doses. Based on the wide margin of safety shown in the major species, the conclusion can be extrapolated to minor poultry species (for fattening or reared for laying/breeding) and growing minor porcine species at 48,000 U/kg feed.

The use of Hemicell® HT as a feed additive does not give rise to concerns for consumers.

[47] 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.

Hemicell® HT and Hemicell® HT-L are not irritant to the skin and eyes; Hemicell® HT is a skin sensitiser. No specific data were provided on the effects on the respiratory system; however, considering the nature of the active substance the additive is considered a potential respiratory sensitiser.

The active substance of the additive is a protein, and as such it will be degraded/inactivated during passage through the digestive tract of the animals or in the environment. However, since uncertainty remains on the absence of the production strain and on the absence of recombinant DNA in the product, the Panel cannot conclude on the environmental safety of the product.

The additive has a potential to be efficacious in chickens for fattening at 32,000 U/kg feed and at 48,000 U/kg feed in turkeys for fattening and weaned piglets. These conclusions can be extended to chickens reared for laying and turkeys reared for breeding at the corresponding doses. The conclusions reached in chickens for fattening can be extrapolated to minor poultry species for fattening or reared for laying/breeding. The Panel cannot conclude on the efficacy of the product in pigs for fattening or in minor porcine species.

Documentation provided to EFSA

1) Hemicell® HT for poultry and pigs. January 2014. Submitted by Eli Lilly and Company Ltd.
2) Hemicell® HT for poultry and pigs. Supplementary information. November 2015. Submitted by Eli Lilly and Company Ltd.
3) Hemicell® HT for poultry and pigs. Supplementary information. September 2016. Submitted by Eli Lilly and Company Ltd.
4) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Hemicell® HT.
5) Comments from the Member States.

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Abbreviations

ANOVA	analysis of variance
ATCC	American Type Culture Collection
bw	body weight
CFU	colony-forming unit
CHO	Chinese hamster ovary
CV	coefficient of variation
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EC	European Commission
EURL	European Union Reference Laboratory
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
LSD	least significant difference
ME	metabolisable Energy
MLLNA	murine local lymph node assay
OECD	Organisation for Economic Co-operation and Development
PCR	polymerase chain reaction
xgl	xyloglucanase gene

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Hemicell® HT

In the current application authorisation is sought under article 4(1) for endo-1,4-β-mannanase (EC 3.2.1.78), under the category/functional group 4(a) 'zootechnical additives/digestibility enhancers', according to the classification system of Annex I of Regulation (EC) No 1831/2003. The feed additive is already authorised under the Commission Regulation (EC) No 786/2007 for chickens for fattening and Commission Implementing Regulation (EU) No 103/2013. According to the Applicant, the feed additive contains the active substance endo- 1,4-β-mannanase (EC 3.2.1.78) produced by the *Paenibacillus lentus* strain.

The feed additive is intended to be marketed as solid (Hemicell® HT) or liquid (Hemicell® HT-L) enzyme preparations, containing a minimum endo-1,4-β-mannanase activity of 1.6 × 108 U/kg and 5.9 × 108 U/L, respectively. According to the Applicant, the activity of endo-1,4-β-mannanase is expressed in units (U), where 1 U is the amount of endo- 1,4-β-mannanase enzyme which liberates 0.72 micrograms of reducing sugars (mannose equivalents) per minute from mannan-containing substrate (locust bean gum) at pH 7.0 and 40°C. Specifically, authorisation is sought for the use of Hemicell®HT and Hemicell® HT-L for a variety of chickens, turkeys and pig species. The feed additive is intended to be used directly into feedingstuffs or through premixtures (Hemicell® HT only) at a recommended minimum endo-1,4-β-mannanase activity of 32,000 U/kg complete feedingstuffs.

For the quantification of the endo-1,4-β-mannanase activity in the feed additive and feedingstuffs, the Applicant submitted two single-laboratory validated and further verified colorimetric methods, based on the reaction of reducing sugars (mannose equivalent) released by the action of endo-1,4-β-mannanase on a mannan-containing substrate (locust bean gum, LBG) in the presence of 3,5-dinitrosalicylic acid (DNS).

The following performance characteristics of the methods were reported in the frame of validation and verification studies for endo-1,4-β-mannanase activity in the feed additive and feedingstuffs ranging from 245 to 690 and 0.015 to 10 mega ($\times 10^6$) units (MU)/kg, respectively: - a relative standard deviation for repeatability (RSDr) ranging from 0.7% to 12.4%; - a relative standard deviation for intermediate precision (RSDip) ranging from 4.2% to 12.4%; and - a recovery rate (RRec) ranging from 91% to 126%. Furthermore, the Applicant reported the limit of quantification (LOQ) of 15,000 U/kg feedingstuffs, which is below the minimum activity recommended by the Applicant in the conditions of use.

For the quantification of endo-1,4-β-mannanase activity in premixtures the Applicant diluted premixture samples with blank feed and applied the above mentioned method for feedingstuffs. Two vitamin/mineral premixtures for poultry and swine were analysed in the frame of homogeneity studies and a relative standard deviation for repeatability (RSDr) ranging from 15.8% to 17.2% was reported for endo-1,4-β-mannanase activity ranging from 50 to 59 MU/kg premixtures.

Based on the performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified colorimetric methods, based on the enzymatic hydrolysis and the reaction of reducing sugars (mannose equivalent) with 3,5- dinitrosalicylic acid (DNS), for the quantification of total endo-1,4-β-mannanase activity 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) is not considered necessary.