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Safety and efficacy of Hemicell®-L (endo-1,4-β-mannanase) as a feed additive for chickens for fattening or reared for laying, turkeys for fattening or reared for breeding and minor poultry species

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Abstract

The additive Hemicell®-L is a liquid preparation of endo-1,4-β-mannanase that is authorised as a zootechnical feed additive for chickens for fattening. The applicant has requested the renewal of the authorisation, new uses and the modification of the manufacturing process including the change of the production strain. The new production strain is a genetically modified strain of *Paenibacillus lentus* obtained from a strain that has been evaluated previously by EFSA and considered to be safe. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) evaluated the genetic modification and concluded that the sequences introduced to obtain the production strain did not raise safety concerns. The production strain and its DNA were not detected in the additive. The Panel concluded that the additive is safe for chickens for fattening and turkeys for fattening at the recommended level. These conclusions were extended or extrapolated to the other target species under application. The Panel concluded that the results obtained in the toxicological studies performed with the fermentation products of the recipient strain and of another genetically modified strain obtained from the same recipient strain could support the safety for the consumer of the product obtained with the new production strain. The additive is not a dermal or eye irritant and it is not a dermal sensitiser. However, considering that the active substance is a protein, the additive should be regarded as a potential sensitiser. The Panel concluded that the additive has a potential to be efficacious as a zootechnical additive for the target species at 79,200 U/kg.

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

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Summary

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 Hemicell®-L (endo-1,4-β-mannanase) as a feed additive for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding and minor poultry species for growing or raised for laying/breeding.

The additive Hemicell®-L is a liquid preparation of endo-1,4-β-mannanase (Enzyme Commission number: 3.2.1.78) that is authorised as a zootechnical feed additive for chickens for fattening. The applicant has requested the renewal of the authorisation, new uses (chickens reared for laying turkeys and minor poultry species) and the modification of the manufacturing process including the change of the production strain. The new production strain is a genetically modified strain of *Paenibacillus lentus* which has been developed from a strain that has been evaluated previously by the European Food Safety Authority (EFSA) and considered to be safe. The genetic modification was fully described and the sequences introduced to obtain the production strain did not raise safety concerns. The production strain and its DNA were not detected in the additive.

Based on the tolerance trials evaluated, the FEEDAP Panel concluded that the additive is safe for chickens for fattening and turkeys for fattening at the recommended level. These conclusions were extended to chickens reared for laying and turkeys reared for breeding. Considering the margin of safety the Panel also extrapolated the conclusion from those studies to minor poultry species for growing or raised for laying/breeding purposes.

Toxicological tests performed with the fermentation product obtained with the new production strain were not made available. However, the applicant submitted two sets of toxicological studies, including genotoxicity and subchronic oral toxicity studies, performed with the fermentation product obtained from the recipient strain or from a genetically modified strain obtained from the same recipient strain. These tests had already been assessed by EFSA in previous assessments. The results of the tests did not indicate a toxicological concern from any of the fermentation products. The Panel evaluated the suitability of those data to support the safety of the product obtained from the new production strain. The Panel concluded that the results obtained in the toxicological studies performed with the fermentation products of the recipient strain and of another genetically modified strain obtained from the same recipient strain could support the safety for the consumer of the product obtained with the new production strain. The additive under assessment was therefore considered safe for consumers of food products obtained from the new production strain.

The additive is not a dermal or eye irritant and it is not a dermal sensitiser. However, considering that the active substance is a protein, the additive should be regarded as a potential sensitiser.

The efficacy of the additive for chickens for fattening has been evaluated in a previous opinion. In that evaluation, four studies were assessed in which the additive used was the previous formulation of the additive obtained with the previous production strain. The results of the four studies showed a significant improvement of body weight gain in four trials and on the feed to gain ratio at the recommended level (79,200 U/kg feed). The use of the previous formulation of the additive still allowed a conclusion for the additive under evaluation because [redacted] the differences in the composition of the additive are not expected to have an impact on the efficacy of the enzyme. Four studies in turkeys for fattening were provided, only one performed with the enzyme produced with the new strain. The results of four long-term trials would allow a conclusion at 100,000 U/kg feed; however, considering the data on the chickens for fattening, the Panel concluded that the additive has a potential to be efficacious in turkeys for fattening at 79,200 U/kg feed. The results from the efficacy trials in chickens were extended to chickens raised for laying and to turkeys reared for breeding purposes. Since the mode of action of enzymes is well known and can reasonably be expected to be the same among poultry species. Therefore, the Panel considers that the conclusions reached in chickens for fattening can be extrapolated to all minor poultry species for growing or reared for laying/breeding purposes.

Table of contents

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. Article 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States. Moreover, Article 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest one year before the expiry date of the authorisation.

The European Commission received two request from Eli Lilly and Company Ltd[2] for authorisation/ re-evaluation/renewal of the authorisation of the product Hemicell®-L (endo-1,4-β-mannanase), when used as a feed additive for chickens for fattening or reared for laying, turkeys for fattening or reared for breeding and minor poultry 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), under Article 13(3) (modification of the authorisation of a feed additive), and under Article 14(1) (renewal of the authorisation). EFSA received directly from the applicant the technical dossiers in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 14 April 2016.

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

1.2. Additional information

The additive Hemicell®-L is a liquid preparation of endo-1,4-β-mannanase (Enzyme Commission number: 3.2.1.78) that is authorised as a feed additive for chickens for fattening.[3] This authorisation is given to the enzyme produced by a non-genetically modified strain of *Bacillus lentus* deposited with the accession number ATCC 55045.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) adopted an opinion on the safety and efficacy of Hemicell®-L as a feed additive for chickens for fattening (EFSA, 2006).

The applicant has requested the renewal of the authorisation and new uses (chickens reared for laying turkeys and minor poultry species), the applicant also requests for a modification of the manufacturing process including the change of the production strain.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of two technical dossiers[4] in support of the authorisation request for the use of Hemicell®-L as a feed additive. 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, Priestley Road, RG24 9 NL Basingstoke, UK.
[3] Commission Regulation (EC) No 786/2007 of 4 July 2007 concerning the authorisation of endo-1,4-β-mannanase EC 3.2.1.78 (Hemicell) as a feed additive OJ L, 5.7.2007, p. 8.
[4] FEED dossier reference: FAD-2015-0042 and FAD-2015-0047.

technical dossiers were prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[5] and the applicable EFSA guidance documents.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.[6]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Hemicell®-L 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 on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2013), 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), 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 (EFSA, 2008c), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012d), Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

This opinion deals with the assessment of the safety and efficacy of Hemicell®-L (endo-1,4-β-mannanase (mannanase); Enzyme Commission number 3.2.1.78) as a feed additive for chickens for fattening or reared for laying, turkeys for fattening or reared for breeding and minor poultry species.

3.1. Characterisation

3.1.1. Characterisation of active substance

The authorisation of the mannanase was given to the mannanase produced by *Bacillus lentus* ATCC 55045. In the current application, the applicant has requested to modify the manufacturing process, including the change of the production strain. The new strain is a genetically modified strain derived from the previous production strain, currently identified as *Paenibacillus lentus*, which is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ) with the accession number DSM 32052.[7]

3.1.1.1. Characterisation of the genetically modified production strain

Characteristics of the recipient or parental microorganism

[redacted]

Characteristics of the donor organisms

[redacted]

[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.

[6] The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2011-0049?search&form-return.

[7] Technical dossier/Section II/Annex II.2.1.2.2.

[redacted]

Description of the genetic modification process

[redacted]

[redacted]

3.1.2. Manufacturing process

[redacted]

3.1.3. Characterisation of the additive

Hemicell®-L is a liquid additive; the composition of which additive has been modified with respect to that previously evaluated, [redacted]. The previous formulation included the enzyme concentrate, sorbitol, monosodium glutamate, sodium chloride and calcium chloride. The new formulation of the additive contains (w/w) the enzyme product (10–30%) sorbitol (30–40%), sodium chloride (6–10%), monosodium glutamate (2–6%), calcium chloride (< 1%), sodium acetate (< 1%), caramel colour (< 1%), potassium sorbate (< 1%), added water (10–50%).[13] This formulation ensures a minimum of 7.2×10^5 Units[14] per mL. The study of the batch-to-batch variation in five batches showed a mean value of 10.7×10^5 Units with a coefficient of variation (CV) of 2.8%. The product has a viscosity of 11.0 centipoises at 20°C, surface tension of 53 dynes/cm,[15] a pH of 7.2 and a specific gravity of 1.23 g/mL.[16]

Three batches of Hemicell®-L were analysed for chemical and microbiological contamination.[17] The analyses of chemical contamination included arsenic (< 0.5 mg/kg), cadmium (< 0.25 mg/kg), lead (< 0.05 mg/kg), mercury (< 0.1 mg/kg), fluoride (< 15 mg/kg), antimony (< 0.5 mg/kg), bismuth (< 1.0 mg/kg), copper (< 0.5 mg/kg), molybdenum (< 0.5 mg/kg), silver (< 0.5 mg/kg) and tin (< 0.5 mg/kg). Polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) were analysed in three batches and were < 0.29 WHO-PCDD-TEQ ng/kg, and < 0.18 WHO-PCDF-TEQ ng/kg, respectively. Analytical data were provided for the following mycotoxins: aflatoxins B1, B2, G1 and G2 (individual < 1 μg/kg), zearalenone (< 25 μg/kg), ochratoxin A (< 1.1 μg/kg), deoxynivalenol (< 0.5 mg/kg), fumonisins (sum of fumonisin B1 and B2 < 1.0 mg/kg) and T2-toxin (< 25 μg/kg).

[redacted] No viable cells or DNA of the production strain were found in the liquid intermediate, since this is a more concentrated product than the additive, the results apply to the additive.

[redacted]

[14] One Unit of mannanase activity is defined as the amount of enzyme that generates 0.72 microgram of reducing sugars from a mannan-containing substrate (locust bean gum) per minute at pH 7.5 and 40°C.

[15] Technical dossier/Section II/Annex II.1.5.1.

[16] Technical dossier/Supplementary information December 2017/Annex II.2.2.1.

[17] Technical dossier/Section II/Annex II.1.4.1.2.

[redacted]

[redacted]

3.1.4. Stability and homogeneity

The shelf life of three batches of Hemicell®-L were studied at −20, 5, 25, 30 and 40°C in samples stored in closed containers for up to 12 months (up to 3 months at 40°C). The initial mean mannanase content was 11 × 10^5 Units/mL and the mean recovery of the initial enzyme activity after 3 months was 100%, 100%, 90%, 80% and 40% for at −20, 5, 25, 30 and 40°C, respectively. The recoveries after 12 months were 101%, 97%, 51% and 24% for samples stored at −20, 5, 25 and 30°C, respectively.[21]

Three batches of the additive were sprayed onto mash or crumble feed for chickens for fattening at an intended level of 79,200 U/kg feed (analysed activity varied from 115,000 to 150,000 U/kg feed).[22] Samples of the feeds were stored in closed containers at −20, 25 and 30°C for up to 3 months and at 40°C for 1 month. Recovery values of the initial activity at the end of the study period were 100%, 90%, 80% and 77% for −20, 25, 30 and 40°C, respectively.

Ten subsamples of each batch of the feed used for the stability study were analysed after the additive was added to study the capacity of the additive to homogeneously distribute. The CV was below 8% in all cases.

3.1.5. Conditions of use

The additive is to be used in feed for chickens for fattening or reared for laying, turkeys for fattening or reared for breeding and minor poultry species at the level of 79,200 U/kg feed.

3.2. Safety

3.2.1. Safety of the genetic modification of the production strain

[redacted]

The production strain and its DNA were not detected in the product. The product Hemicell®-L, manufactured with the production strain *P. lentus* DSM 32052, does not give rise to safety concern with regard to the genetically modified production strain.

3.2.2. Safety for the target species

3.2.2.1. Safety for chickens for fattening

A total of 720 one day-old male chicks (Ross 708) were distributed in groups of 12 birds per pen and allocated to three dietary treatments (20 replicates per treatment).[23] During the study, three basal diets were used according to the birds' requirements. The three basal diets based on maize and soya bean meal were supplemented with Hemicell®-L to provide 0, 100,000 (1.3×) or 1,500,000 (19×) U mannanase/kg feed (confirmed by analysis). Diets were offered in crumble/pelleted form for 35 days. Animal health was monitored daily. Feed intake, body weight and mortality were recorded and feed to gain ratio was calculated (on a pen basis). Necropsy was performed on animals that died during the study. On day 35, one bird per pen was randomly chosen and blood sampled. Blood samples were analysed for biochemical[24] and haematological[25] parameters. A one-way analysis of variance (ANOVA)

[redacted]

[21] Technical dossier/Supplementary information December 2017/Annex II.4.1.3.
[22] Technical dossier/Supplementary information December 2017/Annex II.4.1.4.
[23] Technical dossier/Section III/Annexes III.1.1.1 and III.1.1.1.a.
[24] Including albumin, albumin:globulin ratio, alkaline phosphatase, creatin phophokinase, total protein, chloride, cholesterol, globulin, glucose, potassium, sodium, triglycerie, uric acid, alanine transaminase, aspartate aminotransferase.
[25] Haematocrit, haemoglobin, white blood cells including the differential.

was performed with the data and group means were compared with Dunnett's test. For mortality data, a generalised linear mixed model for binomial data was performed.

Mortality rates were 5.8%, 5.8% and 5.4% for 0, 100,000 and 1,500,000 U/kg feed, respectively, and were not related to the treatment. Daily feed intake (g/day) was 102, 105 and 103 in the groups fed with 0, 100,000 and 1,500,000 U/kg feed, respectively; final body weight (g/bird) was 2,300, 2,361 and 2,355, respectively, and feed to gain ratio was 1.52,1.53 and 1.53, respectively. Blood parameters did not significantly differ in the different dietary groups with the exception of the basophil counts and per cent, where the 1.3× group showed values significantly lower than the control but no differences were found between 19× and control; therefore, this finding is considered of no concern. The supplementation of the experimental diets with Hemicell®-L at 19× the proposed level did not have a negative effect on the performance of the birds.

3.2.2.2. Safety for turkeys for fattening

A total of 315 day-old male turkeys (Nicholas 85) were housed in pens with seven birds per pen and allocated to three dietary treatments (15 replicates per treatment).[26] During the 42 day study, two basal diets (days 0–21; days 21–42) were used according to the birds' requirements which were based on wheat and soya bean meal. The diets were either not supplemented or supplemented with Hemicell®-L to provide 100,000 (1.3×) or 1,500,000 (19×) U/kg feed (confirmed by analysis). Diets were offered in mash form for 42 days and contained monensin. Feed intake, body weight and mortality were recorded and feed to gain ratio was calculated. Necropsy was performed on animals that died during the study. On day 42, blood samples were collected from one bird per pen and analysed for biochemical[27] and haematological[25] parameters. A one-way ANOVA was performed with the data and group means were compared with Dunnett's test; for mortality data, a generalised linear mixed model for binomial data was applied.

Mortality rate was 3.8% in all groups. Daily feed intake (g/day) was 95, 92 and 97 in the control, 100,000 and 1,500,000 U/kg feed groups, respectively; final body weight (kg bird) 2.61, 2.58 and 2.70, respectively, and feed to gain ratio was 1.58, 1.56, 1.57, respectively. In general, blood parameters did not show significant differences in the different dietary groups with the exception of haemoglobin and potassium. Haemoglobin was higher in the groups receiving the mannanase compared to control and the 100,000 U/kg group showed a higher potassium concentration compared to the other groups. These modifications are considered of no concern. Therefore, supplementation of the experimental diets with Hemicell®-L at up to 19× the proposed level did not have a negative effect on the performance of the birds.

The applicant also provided another tolerance trial in turkeys for fattening performed with the mannanase obtained from a precursor strain (holding the same mannanase gene but fewer copies).[28] The data from that study showed no adverse effects after feeding turkeys the mannanase for 91 days and up to a 100-fold the proposed level.

3.2.2.3. Conclusions on safety for the target species

Chickens for fattening and turkeys for fattening can tolerate well up to 19-fold the recommended level of the additive. Therefore, the Panel concludes that the additive is safe at the recommended level of 79,200 U/kg feed for chickens and turkeys for fattening. This conclusion is extended to chickens reared for laying and turkeys reared for breeding.

Considering the margin of safety shown in the tolerance trials, the FEEDAP Panel concludes that the additive is safe for minor poultry species for fattening or reared for laying/breeding up to the point of lay at 79,200 U/kg feed.

3.2.3. Safety for the consumer

No studies were provided with the enzyme concentrate obtained from the production strain subject of the current application in this application [redacted]

[26] Technical dossier/Supplementary information December 2017/Annex III.1.1.1.1.
[27] Including albumin, albumin:globulin ratio, alkaline phosphatase, creatine phosphokinase, chloride, cholesterol, globulins, glucose, potassium, aspartate aminotransferase, sodium total protein, triglyceride, uric acid, BUN, creatinine, total bilirubin, alanine aminotransferase and gamma-glutamil transpeptidase.
[28] Technical dossier/Section III/Annex III.1.1.1 and Supplementary information December 2017.

[redacted] comprised the following tests: a bacterial reverse mutation assay [redacted] performed in compliance with OECD Guideline 471,[29,30] a chromosome aberration test [redacted] in compliance with OECD Guideline 473,[31] an in vitro mammalian cell micronucleus test done with the fermentation product [redacted] in compliance with OECD Guideline 487[32] and a subchronic oral toxicity study for each strain performed according to OECD Guideline 408.[33,34]

The results of the genotoxicity studies showed no evidence for a genotoxicity effect of the test item and the subchronic oral toxicity studies showed no treatment related effects in any of the parameters evaluated.

The Panel in the present assessment evaluated if the available data could support the safety of the product obtained with the newly proposed production strain and manufacturing process. To this scope, three aspects were considered: the differences of the production strains, the manufacturing process and the test items tested.

[redacted]

[29] Technical dossier/Supplementary information December 2017/Annex III.2.2.2.3.
[30] Technical dossier/Supplementary information December 2017/Annex III.2.2.2.1.1.
[31] Technical dossier/Supplementary information December 2017/Annex III.2.2.2.4.
[32] Technical dossier/Supplementary information December 2017/Annex III.2.2.2.2.1.
[33] Technical dossier/Section III/Annex 2.2.3.
[34] Technical dossier/Section III/Annex 2.2.4.

[redacted]

3.2.3.1. Conclusions on safety for the consumer

Hemicell®-L obtained from the fermentation product from *P. lentus* DSM 32052 is considered not to raise concerns for the consumers of food products derived from animals fed with the additive Hemicell®-L.

3.2.4. Safety for the user

3.2.4.1. Effects on the respiratory tract

No specific studies have been provided. Enzymes are considered as potential respiratory sensitisers.

3.2.4.2. Effects on the skin and eyes

An acute dermal irritation study (OECD Guideline 404)[35] and an eye irritation study (OECD Guideline 405)[36] performed in male New Zealand White albino rabbits were done with Hemicell®-L. The results showed that the test material is non-irritant to skin or eye, respectively.

The potential of Hemicell®-L to cause or elicit skin sensitisation reactions (allergic contact dermatitis) was assessed via the murine local lymph node assay (MLLNA), in compliance with OECD guideline 429.[37] A total of 35 female CBA/J mice were used in this study. Animals treated with Hemicell®-L showed sensitisation index below 3 which indicates no sensitisation. The positive control group resulted in a stimulation index of 4.6.

3.2.4.3. Conclusions on safety for the user

The additive is not a dermal or eye irritant and it is not a dermal sensitiser. However, considering that the active substance is a protein, the additive should be regarded as a potential respiratory sensitiser.

3.2.5. Safety for the environment

The production strain and its DNA were not detected in the final product. The final product does not pose any environmental safety concern associated with the genetic modification. 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. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

3.3. Efficacy

The applicant submitted efficacy trials in chickens and turkeys. Only one trial in turkeys for fattening (trial 4) was conducted with the product obtained with the new production strain. [redacted] no differences would be expected regarding the efficacy of the main enzyme activity obtained with the new production strain.

[redacted]

[35] Technical dossier/Section III/Annex III.3.1.2.1.
[36] Technical dossier/Section III/Annex III.3.1.2.2.
[37] Technical dossier/Section III/Annex III.3.1.2.3.

[redacted] Therefore, the studies conducted with the product obtained following the previous manufacturing process are considered relevant for this assessment.

3.3.1. Efficacy for chickens for fattening

The efficacy studies provided to support the efficacy of the additive for chickens for fattening has been evaluated in a previous opinion (EFSA, 2006). The results of the four studies showed a significant improvement of body weight gain in four trials and on the feed to gain ratio at the recommended level (79,200 U/kg feed).

3.3.2. Efficacy for turkeys for fattening

A total of five studies were assessed. One of the studies was not considered further due to the high mortality registered during the study (above 10%).[41]

In the first trial, a total of 480 day-old female turkeys (Nicholas) were distributed in pens in groups of 15 birds and allocated to four dietary treatments (eight replicates per treatment).[42] The birds were offered a total of four phase diets according to the birds' requirements. The study followed a 2 × 2 design with two basal diets and two levels of enzyme supplementation. Two treatments were obtained from a standard basal diets (St), according to birds requirements, based on maize and soybean meal that were either not supplemented (control) or supplemented with mannanase to provide 79,200 U/kg feed (analytical confirmation; 8,940 and 113,283 U/kg feed, respectively). The other two dietary treatments were obtained from the standard basal diets with added dried distillers with solubles grains (St + DDGS, 15% inclusion) that were either not supplemented (control) or supplemented with Hemicell®-L to provide 0 or 79,200 U/kg feed (analytical confirmation; 9,623 and 96,925 U/kg feed, respectively). Diets were offered in crumb/pelleted form for 84 days.

In the second trial, two replicated studies were conducted. In each trial, an initial number of 616 day-old male turkeys (Nicholas) were distributed in pens in groups of 22 birds per pen and allocated to four dietary treatments (seven replicates per treatment).[43] The number of birds was reduced to 17 on day 28. The studies followed a 2 × 2 design with two basal diets (with different protein and fibre content) and two levels of enzyme supplementation (0 or 100,000 U/kg feed). Two treatments were obtained from basal diets, according to birds requirements', based on maize and soybean meal (soya bean meal with 48% crude protein) that were either not supplemented (control) supplemented with Hemicell®-L to provide 100,000 U/kg feed (analytical confirmation available only for the second study; 18,960 and 141,320 U/kg feed, respectively). The other two dietary treatments were obtained from the same basal diet that was modified to include soybean meal with 44% of crude protein and soya hulls that was either not supplemented (control) or supplemented with Hemicell®-L 100,000 U/kg feed (analytical confirmation available only for the second study; 14,990 and 145,280 U/kg feed, respectively). Diets were offered in crumb/pelleted form for 126 days. During the study, experimental treatments were offered in a total of five phase diet, according to the birds' requirements.

[redacted]

[41] Technical dossier/Section IV/Annex IV.3.3.
[42] Technical dossier/Section IV/Annex IV.3.1.
[43] Technical dossier/Section IV/Annex IV.3.2.

In the third trial, a total of 760 day-old female turkeys (Nicholas) were distributed in pens in groups of 19 birds and allocated to four dietary treatments (10 replicates per treatment).[44] During the study, the dietary treatments were offered in a total of five phase diet, according to the birds' requirements. The studies followed a 2 × 2 design with two basal diets with two different energy contents and two levels of enzyme supplementation (0 or 79,200 U/kg). Two treatments were obtained from basal diets (from starter to finisher) containing a very low energy content (VLEC, 11.85–13.85 MJ ME/kg feed) that was either not supplemented (control) or supplemented with Hemicell®-L to provide 79,200 U/kg feed (confirmation: 10,800 and 120,560 U/kg feed). The other two dietary treatments were obtained from a diet formulated to contain 0.25 MJ ME/kg feed more compared to VLEC, low energy content (LEC) that was either not supplemented (control) or supplemented with Hemicell®-L 79,200 U/kg feed (confirmation: 12,800 and 120,640 U/kg feed). Diets were offered in mash/pelleted form for 98 days.

In the fourth trial, a total of 480 day-old female turkeys (BUT10) were distributed in pens in groups of 20 birds and pens were allocated to two dietary treatments (representing 12 replicates per treatment).[45] During the study, the dietary treatments were offered in a total of five phase diet, according to the birds' requirements. The basal diets were either not supplemented (control) or supplemented with Hemicell®-L to provide 100,000 U/kg feed (confirmation below limit of quantification (LOQ) and 130,000 U/kg feed). Feed was offered in pelleted form during 3 weeks, later on it was offered in crumbles up to week 12 of life.

During the studies, mortality and general health of the birds was monitored. Feed intake and body weight of the birds were measured throughout the study and feed to gain was calculated.

An ANOVA was done with the data from each study, separately. The experimental units were the pens. The model used included the treatment in all trials; in trial 2, the study, the basal diet, the enzyme and their interactions were considered. Mean groups were compared using the Tukey test. The results of the studies are presented in Table 1.

Table 1: Effect of Hemicell®-L on the performance of turkeys for fattening

Trial	Basal diet	Intended level (U/kg)	Feed intake (kg)[1]	Body weight[2] (kg)	Feed to gain ratio	Mortality (%)
1[3]	St	0	16.86^{a}	7.72^{ab}	2.19^{b}	4.17
	St	79,200	16.12^{ab}	7.89^{a}	2.04^{a}	0.83
	St + DDGS	0	16.52^{ab}	7.51^{b}	2.20^{b}	1.67
	St + DDGS	79,200	15.66^{b}	7.56^{b}	2.07^{ab}	0.83
2[4]	SBM 48	0	41.65	14.91	2.70	8.08
	SBM 48	100,000	41.00	15.03	2.63	5.06
	SBM 44	0	41.81	14.39	2.79	2.88
	SBM 44	100,000	41.25	14.77	2.70	4.74
3[5]	LEC	0	20.25^{b}	9.24^{b}	2.17^{ab}	2.1
	LEC	79,200	20.52^{ab}	9.56^{a}	2.13^{b}	1.6
	VLEC	0	20.38^{b}	9.18^{b}	2.21^{a}	1.1
	VLEC	79,200	21.11^{a}	9.55^{a}	2.19^{a}	2.1
4	–	0	0.209	8.02	2.20^{a}	0
	–	100,000	0.204	8.05	2.15^{b}	1.25

[1] Values are total feed intake in trials 1, 2 and 3, and daily feed intake in trial 4.
[2] Values are body weight gain in trials 1 and 3, and body weight in trials 2 and 4.
[3] St: maize and soybean meal-based diet, St + DDGS: St + 15% dried distillers grain with solubles
[4] SBM 48: soya bean meal crude protein 48% and maize diet, SBM 44: soya bean meal crude protein 44% and maize diet. The diets with SBM 48 showed higher values of protein and lower values of fibre compared to the SBM 44%. The mean values for the effect of the enzyme are not provided in the report.
[5] VLEC: very low energy content diet formulated to have 11.85–13.85 MJ/kg feed from starter to finisher diets; LEC: low energy content diet formulated to have 0.25 MJ/kg feed more than VLEC in each phase.
a,b, values within a column and within a study with different superscript are significantly different ($p < 0.05$).

Mortality in the trials was not different between the treatments. In trial 1, the birds receiving the enzyme at the recommended level in a standard diet showed a better feed to gain ratio compared to

[44] Technical dossier/Section IV/Annex IV.3.4.
[45] Technical dossier/Supplementary information December 2017/Annex IV.3.5.

control. In trial 2, the results showed no interactions between the basal diet and the addition of the enzyme. The birds receiving the enzyme at 100,000 U/kg feed, regardless of the basal diet, showed significantly lower feed intake, higher body weight and consequently a better feed to gain ratio. In trial 3, birds receiving the enzyme at the recommended level showed a higher body weight, regardless of the basal diet considered. In trial 4, birds receiving the enzyme at 100,000 U/kg feed showed a better feed to gain ratio.

The Panel notes that the data provided with turkeys for fattening would support the efficacy at 100,000 U/kg feed. However, considering the provisions of the Guidance on the assessment of efficacy for feed additive (EFSA FEEDAP Panel, 2017), the Panel would extrapolate the efficacious level in chickens for fattening (79,200 U/kg feed) to turkeys for fattening.

3.3.2.1. Conclusions on efficacy for the target species

The Panel concludes that the additive has potential to be efficacious as a zootechnical additive in chickens for fattening and turkeys for fattening at 79,200 U/kg feed. The Panel considers that these conclusions apply to chickens reared for laying and turkeys reared for breeding at the corresponding levels.

The effect of the additive can reasonably be expected to be the same among poultry species. Therefore, the Panel considers that the conclusions reached in chickens for fattening can be extrapolated to all minor poultry species for growing or reared for laying/breeding purposes.

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[46] and Good Manufacturing Practice.

4. Conclusions

Viable cells and the DNA of the production strain were not found in the intermediate product used to formulate the additive. The product Hemicell®-L, manufactured with the production strain *P. lentus* DSM 32052, does not give rise to safety concern with regard to the genetically modified production strain.

The additive is safe at the recommended level for chickens and turkeys for fattening. This conclusion is extended to chickens reared for laying and turkeys reared for breeding and extrapolated to minor poultry species for growing or reared for laying/breeding purposes.

The additive obtained from *P. lentus* DSM 32052 raises no safety concerns for the consumer of food products derived from animals receiving the additive.

The additive is not a dermal or eye irritant and it is not a dermal sensitiser. However, considering that the active substance is a protein, the additive should be regarded as a potential respiratory sensitiser.

The use of the product as a feed additive raises no concerns for the environment.

The additive has a potential to be efficacious as a zootechnical additive in chickens for fattening or reared for laying, turkeys for fattening and reared for breeding purposes, minor poultry species for growing or reared for laying/breeding purposes at 79,200 U/kg.

Documentation provided to EFSA

1) Hemicell®-L for chickens. December 2015. Submitted by Eli Lilly and Company Ltd.
2) Hemicell®-L for turkeys. December 2015. Submitted by Eli Lilly and Company Ltd.
3) Hemicell®-L for chickens. Supplementary information. December 2017. Submitted by Eli Lilly and Company Ltd.
4) Hemicell®-L for turkeys. Supplementary information. December 2017. Submitted by Eli Lilly and Company Ltd.
5) Comments from Member States.

[46] 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.

Chronology

Date	Event
26/11/2015	FAD-2015-0042 Dossier received by EFSA
21/12/2015	FAD-2015-0047 Dossier received by EFSA
11/12/2015	FAD-2015-0042 Reception mandate from the European Commission
19/01/2016	FAD-2015-0047 Reception mandate from the European Commission
14/04/2016	Applications validated by EFSA – Start of the scientific assessment
09/06/2016	FAD-2015-0047 Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety*
13/06/2016	FAD-2015-0042 Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety*
14/07/2016	Comments received from Member States for the two applications
21/12/2017	Reception of supplementary information from the applicant for the two applications - Scientific assessment re-started
26/04/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 for the two applications – Scientific assessment suspended *Issues: efficacy*
24/01/2019	Reception of supplementary information from the applicant for the two applications - Scientific assessment re-started
26/02/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ANOVA	analysis of variance
ATCC	American Type Culture Collection
CFU	colony forming units
CV	coefficient of variation
DSMZ	German Collection of Microorganisms and Cell Cultures
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
LEC	low energy content
LOQ	limit of quantification
MLLNA	murine local lymph node assay
OECD	Organisation for Economic Co-operation and Development
PCDD	polychlorinated dibenzo-*p*-dioxins
PCDF	polychlorinated dibenzofurans
PCR	polymerase chain reaction
TEQ	toxic equivalent
VLEC	very low energy content
WHO	World Health Organization