SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of endo-1,4-β-D-mannanase produced by *Paenibacillus lentus* DSM 33618 (Hemicell® HT/HT-L) for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, minor poultry species to point of lay, pigs for fattening, weaned piglets and minor porcine species (Elanco GmbH)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of endo-1,4-β-D-mannanase (Hemicell® HT/HT-L) produced by a genetically-modified strain of *Paenibacillus lentus* (DSM 33618) as a zootechnical feed additive for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, minor poultry species to point of lay, pigs for fattening, piglets (weaned) and minor porcine species. The production strain was obtained from a *Paenibacillus lentus* recipient strain that has been evaluated previously by EFSA and considered to be safe. The genetic modification does not raise safety concerns and there were no antibiotic resistance genes from the genetic modification in the production strain. Viable cells and the DNA of the production strain were not found in the intermediate product used to formulate the additive. Hemicell® HT/HT-L produced by *Paenibacillus lentus* DSM 33618 is considered safe for the above-mentioned target species at the intended conditions of use. The use of Hemicell® HT/HT-L as a feed additive raises no concerns for the consumer or for the environment. Hemicell® HT/HT-L is not irritant to the skin and eyes but is regarded as a dermal sensitiser and a potential respiratory sensitiser. The additive has a potential to be efficacious at 32,000 U/kg in chickens for fattening, chickens reared for laying, minor poultry species for fattening or reared for laying/breeding, pigs for fattening and minor porcine species, and at 48,000 U/kg in turkeys for fattening, turkeys reared for breeding and weaned piglets.

Keywords: zootechnical additives, digestibility enhancers, Hemicell® HT/HT-L, endo-1,4-β-D-mannanase, *Paenibacillus lentus* DSM 33618, safety, efficacy

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

The European Commission received a request from Elanco GmbH[2] for the authorisation of the additive consisting of endo-1,4-β-D-mannanase produced by *Paenibacillus lentus* DSM 33618 (Hemicell® HT/HT-L), when used as a feed additive for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, minor poultry species to point of lay, pigs for fattening, weaned piglets and minor porcine species (category: zootechnical additives; functional group: digestibility enhancers).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 11 August 2021.

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 feed additive consisting of endo-1,4-β-D-mannanase produced by *Paenibacillus lentus* DSM 33618 (Hemicell® HT/HT-L), when used under the proposed conditions of use (see **Section 3.1.5**).

1.2. Additional information

The additive Hemicell® HT/HT-L under assessment, consisting of endo-1,4-β-D-mannanase, produced by *P. lentus* DSM 33681 has not been previously assessed as a feed additive by EFSA and is not currently authorised for use in food and feed in the European Union (EU).

The EFSA Panel on Additives and Products or Substances used in Animal feed (FEEDAP) issued two opinions on the safety and efficacy of Hemicell® HT/HT-L produced by a different strain of *Paenibacillus lentus* (DSM 28088) (EFSA FEEDAP Panel, 2017a, 2018a). Hemicell® HT/HT-L consisting of endo-1,4-β-D-mannanase produced by *P. lentus* DSM 28088 is currently authorised as a feed additive for use in chickens for fattening, chickens reared for laying and minor poultry species other than laying birds, turkeys for fattening, turkeys reared for breeding, weaned piglets, pigs for fattening and minor porcine species for fattening (4a29).[3]

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[4] in support of the authorisation request for the use of endo-1,4-β-D-mannanase produced by *P. lentus* DSM 33618 (Hemicell® HT/HT-L) as a feed additive. The dossier was received on 24/3/2021 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00346.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Elanco GmbH, Heinz-Lohmann Str 4, 27,472 Cuxhaven, Germany.
[3] Commission Implementing Regulation (EU) 2018/1565 of 17 October 2018 concerning the authorisation of a preparation of endo-1,4-beta-mannanase produced by *Paenibacillus lentus* (DSM 28088) as a feed additive for chickens for fattening, chickens reared for laying and minor poultry species other than laying birds, turkeys for fattening, turkeys reared for breeding, weaned piglets, pigs for fattening and minor porcine species (holder of authorisation Elanco GmbH) C/2018/6728, OJ L 262, 19.10.2018, p. 24–26.
[4] Dossier reference: FAD-2021-0063.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 13/8/2021 to 12/11/2021 for which received comments that were considered for the assessment.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the endo-1,4-β-D-mannanase in animal feed.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of endo-1,4-β-D-mannanase produced by *P. lentus* DSM 33618 (Hemicell® HT/HT-L) is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017b), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017d), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018c) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

This opinion deals with the safety and efficacy of Hemicell® HT/HT-L, consisting of endo-1,4-β-D-mannanase (IUBMB EC 3.2.1.78; mannanase) produced by *Paenibacillus lentus* DSM 33618, as a zootechnical additive (digestibility enhancer) for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, pigs for fattening, weaned piglets and minor poultry/porcine species.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The mannanase is produced by a genetically modified *Paenibacillus lentus* strain and is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ) with the accession number DSM 33618.[7]

The taxonomic identification of the production strain was confirmed [redacted][8]

The susceptibility of the strain to the antibiotics recommended by the FEEDAP Panel (EFSA FEEDAP Panel, 2018c) was tested by broth microdilution following the Clinical and Laboratory Standards Institute (CLSI) performance standards. All the minimum inhibitory concentration (MIC) values determined were equal to or fell below the corresponding cut-off values for 'other Gram positive'. Therefore, *P. lentus* DSM 33618 is considered susceptible to the relevant antibiotics.[9]

The genome of the production strain was queried for presence of genes coding for or contributing to antimicrobial resistance (AMR) [redacted][10] No hits of concern were identified.

The toxigenic potential of *P. lentus* DSM 33618 was assessed according to the provisions for *Bacillus* spp. of the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018c). No lysis of Vero cells was detected; therefore, the strain is considered to be non-cytotoxic.[11]

[5] Evaluation report received on 29/08/2022 and available on the EU Science Hub https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en.
[6] 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.
[7] Technical dossier/Section II/Annex II_2_1_2_3.
[8] Technical dossier/Section II/Annex II_2_1_2_1.
[9] Technical dossier/Section II/Annex II_2_2_2_4.
[10] Technical dossier/Supplementary information November 2022/Annex II_2_2_2_5.
[11] Technical dossier/Section II/Annex II_2_2_2_1.

[redacted]

3.1.1.1. Characterisation of the genetically modified production strain

[redacted]

Characteristics of the recipient or parental microorganism

[redacted]

Characteristics of the donor organisms

[redacted]

Description of the genetic modification process

[redacted]

[12] Technical dossier/Supplementary information November 2022/Annex II_2_2_2_8.
[13] Technical dossier/Section II/Annex II_2_2_2_6.
[14] Technical dossier/Section II/Annex II_2_2_2_7.

[redacted][15]

3.1.2. Manufacturing process

The manufacturing process is the same as the one reported for the product Hemicell® HT/HT-L produced by strain DSM 28088 and assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2017a). [redacted][16] The resulting product, liquid intermediate, is either mixed with excipients for the preparation of the liquid formulation (Hemicell® HT-L) or spray-dried and then mixed with excipients/carriers for the preparation of the solid formulation (Hemicell® HT) (see Section 3.1.3).

[redacted][17]

3.1.3. Characterisation of the additive

The additive is available in two formulations:

- Hemicell® HT, a solid formulation with a minimum enzymatic activity of 1.60×10^8 Units (U)[18] mannanase per kg product, and
- Hemicell® HT-L, a liquid formulation with a minimum enzymatic activity of 5.90×10^8 U mannanase per L product.

Batch-to-batch variation of the HT form, tested in five batches, showed a mean value of 3.15×10^8 U/kg ranging 2.89×10^8–3.48×10^8 U/kg (coefficient of variation (CV) ranged 8.0%–9.4%).[19] This formulation contains (w/w) linseed meal (58%), calcium carbonate (38%), food-grade mineral oil[20] (1%) and dried fermentation product (3%, spray-dried with maltodextrin).[21] The dusting potential was measured in three batches using the Stauber–Heubach method and ranged 10–15 mg/m^3.[22] Particle size distribution was measured in three lots by laser diffraction analysis and showed a mean particle size ranged 71.08–1,388 μm, particles below 100 μm represented 11.96%–14.70% (v/v), particles below 50 μm represented 5.36%–7.09% (v/v) and particles below 10 μm represented 0.86%–1.11% (v/v).[22] The bulk density of the formulation ranges 740–756 kg/m^3, while the tap density is 877–893 kg/m^3 and solid density 1,720–1,744 kg/m^3.[22]

Batch-to-batch variation of the HT-L form, tested in five batches, showed a mean value of 1.42×10^9 U/L ranging from 1.23 to 1.67×10^9 U/L (CV ranged 9.9%–13.5%).[23] This formulation contains (w/w) sorbitol [49% (70% solution in water)], liquid fermentation product (15%), sodium chloride (8%), monosodium glutamate (4%), sodium acetate (< 0.1%), caramel colour (< 0.1%), potassium sorbate (< 0.1%), calcium chloride (< 0.1%) and added water (23%).[24] Three batches were used to determine the physical state of the liquid formulation, and the following results were obtained with measurements at 25°C: vapour pressure ranges 1.97–2.51 kPa, viscosity ranges 9.99–11.30 cP and the specific weight ranges 12,130–12,160 N/m^3.[25]

The composition of the final formulations is essentially the same[26] as the one reported for the product Hemicell® HT/HT-L produced by strain DSM 28088 and assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2017a).

[15] Technical dossier/Section II/Annex II_2_1_2_1 and Annex II_2_1_2_4.
[16] Technical dossier/Section II/Annex II_3_2_1 and Annex II_3_2_2.
[17] Technical dossier/Supplementary Information November 2022/EFSA SIn Reply FINAL.
[18] One unit is defined as the amount of endo-1,4-β-mannanase enzyme which generates 0.72 microgram of reducing sugars per minute from a mannose containing substrate at pH 7.0 and 40°C.
[19] Technical dossier/Section II/Annex II_1_3_5.
[20] Technical dossier/Supplementary Information November 2022/Annex_II_1_3_1_1.
[21] Technical dossier/Section II/Annex II_1_3_1.
[22] Technical dossier/Section II/Annex II_1_5_1.
[23] Technical dossier/Section II/Annex II_1_3_6.
[24] Technical dossier/Section II/Annex II_1_3_2.
[25] Technical dossier/Section II/Annex II_1_3_4.
[26] Technical dossier/Supplementary Information November 2022/Annex_II_8 and Annex_II_9.

Three batches of each formulation were analysed for chemical contamination.[27] Results for mycotoxins, arsenic and mercury content in both formulations showed values below the limit of detection (LOD) and/or limit of quantification (LOQ) of the analytical methods.[28,29] In the liquid formulation, the values for cadmium and lead were also below the LOQ, while in the solid formulation, cadmium ranged 0.36–0.37 µg/g and lead ranged 0.34–0.40 µg/g.[29] Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs) content were analysed in three batches of each formulation. Results for the solid formulation, PCDD/PCDF ranged 0.40–0.52 ng WHO-PCDD/F-TEQ/kg while PCBs ranged 0.05–0.20 ng WHO-PCB-TEQ/kg. Results for the liquid formulation showed values for both PCDD/PCDF and PCBs below the LOD (0.66 ng/kg).[30] Three batches of each formulations were tested for microbiological contamination, namely for presumptive *Bacillus cereus*, Bile-tolerant Gram-negative bacteria or *Enterobacteriaceae, Salmonella* spp. and yeasts and filamentous fungi.[31] In the solid formulation, presumptive *Bacillus cereus* were found in two batches (100 and 200 CFU/g), while in three batches both filamentous fungi (range 40–65 CFU/g) and bile-tolerant Gram-negative bacteria were detected (with two batches with 1,000 most probable number (MPN)/g and one batch with > 1,000 MPN/g). In addition, results showed that *Salmonella* spp. was not detected in 25 g and yeasts counts were lower than 10 CFU/g. In the three batches of the liquid formulation, *Enterobacteriaceae* were detected < 10 MPN/g, *Salmonella* spp. was not detected in 25 mL, yeasts and filamentous fungi were < 10 CFU/g and presumptive *Bacillus cereus* were < 100 CFU/g.[32]

The detected amounts of the above described impurities do not raise safety concerns.

The presence of the production strain in the product was assessed in three independent batches of the liquid intermediate.[33] [redacted] The [redacted] was 10 ng/mL. No DNA from the production strain was detected in the intermediary product.

The antimicrobial activity of liquid culture supernatants and liquid intermediate used to formulate the additive (three batches each) was tested using the disk diffusion method against reference strains (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 11129, *Bacillus cereus* ATCC 2, *Bacillus circulans* ATCC 4516, *Streptococcus pyogenes* ATCC 12344 and *Serratia marcescens* ATCC 14041). No inhibition was observed, denoting the lack of antimicrobial activity of the test items.[36]

3.1.4. Stability and homogeneity

The shelf-life and stability data provided were those reported for the product Hemicell® HT/HT-L produced by strain DSM 28088 (EFSA FEEDAP Panel, 2017a). The enzyme contained in the additive and the composition of the additive is essentially the same therefore the Panel considers that the results obtain apply to the production strain *P. lentus* DSM 33618.

[27] Technical dossier/Supplementary Information November 2022/Annex II_1_4_1_3.
[28] Limit of detection (LOD): aflatoxin B1 1.3 ppb; aflatoxin B2 1.2 ppb; aflatoxin G1 1.1 ppb; aflatoxin G2 1.6 ppb; Fumonisin B1, Fumonisin B2, Fumonisin B3, deoxynivalenol and acetyldeoxynivalenol 0.1 ppm; Ochratoxin A 1.1 ppb; T-2 toxin and HT-2 toxin 0.2 ppm; zearalenone 51.7 ppb.
[29] Limit of quantification: arsenic 0.50 µg/g, cadmium 0.25 µg/g, lead 0.05 µg/g and mercury 0.10 µg/g.
[30] Technical dossier/Section II/Annex II_1_4_1_3.
[31] Technical dossier/Supplementary Information November 2022/Annex II_1_4_1_3 and Spontaneous submission November 2021/Annex_II_1_4_1_4.
[32] Technical dossier/Spontaneous submission November 2021/Annex_II_1_4_1_4.
[33] Technical dossier/Supplementary Information November 2022/Annex_II_2_1_2_8.
[34] Technical dossier/Section II/Annex II_2_1_2_7.
[35] Technical dossier/Supplementary Information November 2022/EFSA SIn Reply FINAL and Annex II_2_1_2_9.
[36] Technical dossier/Section II/Annex II_2_2_2_3.

3.1.5. Conditions of use

The endo-1,4-β-D-mannanase produced by *P. lentus* DSM 33618 (Hemicell® HT/HT-L) is intended for use in feed for chickens for fattening and reared for laying, pigs for fattening, minor poultry species other than laying birds and minor porcine species for fattening at a proposed minimum use level of 32,000 U/kg feed and for turkeys for fattening and reared for breeding and weaned piglets at the use level of 48,000 U/kg feed.

The minimum concentrations proposed in these conditions of use are the same as for the product currently authorised produced by *P. lentus* DSM 28088.[3]

3.2. Safety

3.2.1. Safety of the genetic modification of the production strain

The production strain *P. lentus* DSM 33618 has been shown to be non-toxigenic, is susceptible to all relevant antibiotics and its genome contains no known AMR genes or genes encoding virulence and pathogenicity factors of concern. No antimicrobial activity was observed against the reference strains tested.

The genes introduced during the genetic modification remaining in the production strain are of no concern. Further, the production strain and its recombinant DNA were not detected in the final product. The additive manufactured using the production strain *P. lentus* DSM 33618, does not give rise to safety concerns with regard to the genetic modification of the production strain.

3.2.2. Safety for the target species, consumer and user

No data obtained with the product manufactured with *P. lentus* DSM 33618 was submitted. The applicant referred to the studies evaluated in the context of the assessment of the mannanase produced by *P. lentus* DSM 28088 (EFSA FEEDAP Panel, 2017a), which included tolerance and toxicological tests to support the safety for the target species, consumers and users.

The manufacturing process and the composition of the additive manufactured using *P. lentus* DSM 33618[37] are essentially the same as the one manufactured with strain DSM 28088 (EFSA FEEDAP Panel, 2017a). Moreover, the difference in the genetic modification between the two strains is not expected to result in a difference in the toxicological profile of the two strains. Consequently, the data obtained with the product manufactured with *P. lentus* DSM 28088 and previously evaluated by the FEEDAP Panel can be used to support the safety for the product manufactured with *P. lentus* DSM 33618.

Based on tolerance trials in chickens and turkeys for fattening, and weaned piglets, 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 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' (EFSA FEEDAP Panel, 2017a).

In the assessment of the safety for the consumer for the product manufactured with *P. lentus* DSM 28088 the Panel evaluated two genotoxicity studies (an Ames test and an *in vitro* micronucleus test, covering gene mutations and chromosomal damage, respectively) and a sub-chronic oral toxicity study. Based on these data, the Panel concluded that 'the results obtained with the fermentation product (used to prepare the additive) in the genotoxicity studies and in the sub-chronic oral toxicity study do not indicate any reason for concern for consumer safety arising from the use of the product as a feed additive' (EFSA FEEDAP Panel, 2017a).

In the assessment of the safety for the user for the product manufactured by *P. lentus* DSM 28088, the Panel has concluded that Hemicell® HT/HT-L is not irritant to the skin and eyes but is regarded as a dermal sensitiser and a potential respiratory sensitiser (EFSA FEEDAP Panel, 2017a). The same conclusions can be extrapolated to the product herein under assessment.

3.2.3. Safety for the environment

The production strain and its DNA were not detected in the additive liquid intermediate. The final product poses no environmental safety concerns associated with the genetic modification. The active

[37] Technical dossier/Section II/Annex II_3_2_2.

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 did not provide new studies with the herein additive produced by the new production strain (*P. lentus* DSM 33618) and referred to the studies submitted for the assessment of the product manufactured by *P. lentus* DSM 28088 (EFSA FEEDAP Panel, 2017a, 2018a). In those assessments, and based on the efficacy studies conducted in chickens and turkeys for fattening, weaned piglets and pigs for fattening, the Panel concluded that the additive has the potential to be efficacious at 32,000 U/kg in chickens for fattening, chickens reared for laying, minor poultry species for fattening or reared for laying/ breeding, pigs for fattening and minor porcine species for fattening, and at 48,000 U/kg in turkeys for fattening, turkeys reared for breeding, weaned piglets and minor porcine species in the weaning period. Although the production strains are different, these share the same mannanase gene, and the activity of the mannanase is expected to be the same with no differences regarding the efficacy of the main enzyme activity. Consequently, the Panel considers that the conclusions drawn in the assessment of the product manufactured with *P. lentus* DSM 28088 apply to the product manufactured with *P. lentus* DSM 33618.

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[38] 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®-HT, manufactured with the production strain *P. lentus* DSM 33618, does not give rise to safety concern regarding the genetically modified production strain.

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 can be extended to chickens reared for laying and turkeys reared for breeding and to pigs for fattening at the corresponding levels. Moreover, it can be extrapolated to minor poultry species (for fattening or reared for laying/breeding) and growing minor porcine species.

The use of Hemicell® HT/HT-L as a feed additive raises no concerns for the consumer and the environment.

Hemicell® HT/HT-L is not irritant to the skin and eyes but is regarded as a dermal sensitiser and a potential respiratory sensitiser.

The additive has a potential to be efficacious at 32,000 U/kg in chickens for fattening, chickens reared for laying, minor poultry species for fattening or reared for laying/breeding, pigs for fattening and minor porcine species for fattening, and at 48,000 U/kg in turkeys for fattening, turkeys reared for breeding, weaned piglets and minor porcine species in the weaning period.

References

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Dierick NA, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M and Cocconcelli PS, 2017a. 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, Turkey for fattening, turkeys reared for breeding, weaned

[38] 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.

piglets, pigs for fattening and minor poultry and porcine species. EFSA Journal 2017;15(1):4677, 22 pp. https://doi.org/10.2903/j.efsa.2017.4677

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Dujardin B, Galobart J and Innocenti ML, 2017b. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pp. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML, 2017c. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 12 pp. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017d. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Dierick NA, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Cocconcelli PS, 2018a. Scientific opinion on the safety and efficacy of Hemicell® HT (endo-1,4-β-mannanase) as a feed additive for chickens for fattening, chickens reared for laying, Turkey for fattening, turkeys reared for breeding, weaned piglets, pigs for fattening and minor poultry and porcine species. EFSA Journal 2018;16(5):5270, 7 pp. https://doi.org/10.2903/j.efsa.2018.5270

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2018b. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018c. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Bastos M, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brock T, de Knecht J, Kolar B, van Beelen P, Padovani L, Tarres-Call J, Vettori MV and Azimonti G, 2019. Guidance on the assessment of the safety of feed additives for the environment. EFSA Journal 2019;17(4):5648, 78 pp. https://doi.org/10.2903/j.efsa.2019.5648

Abbreviations

AMR	antimicrobial resistance
ANI	Average Nucleotide Identity
BMI	Blue Mannanase Indicator
CFU	colony forming unit
CLSI	Clinical and Laboratory Standards Institute
CV	coefficient of variation
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
MPN	most probable number
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzodioxin

PCDF	polychlorinated dibenzofuran
PCR	polymerase chain reaction
TEQ	toxic equivalents
VFDB	Virulence Factor Database
WGS	whole genome sequence
WHO	World Health Organization