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Safety of Endofeed® DC (endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase) as a feed additive for chickens for fattening, laying hens, pigs for fattening and minor poultry and porcine species

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Abstract

Endofeed® DC is a preparation of endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase to be used as a feed additive for chickens for fattening, laying hens, pigs for fattening and minor poultry and porcine species. In a previous assessment, the safety of the additive for the target species, user and environment as well as the efficacy were established. In that assessment, the applicant provided in order to address the safety for the consumer, a bacterial reverse mutation assay and an *in vitro* micronucleus test, and a subchronic oral toxicity study. However, considering the limitations on the description of the manufacturing process, the characterisation of the additive and on the toxicological studies provided, the Panel could not conclude on the safety for the consumer. The applicant provided new data/information on the manufacturing process, characterisation of the additive and new toxicological studies, to support the safety for the consumer, which was the main subject of this opinion. Complete and detailed information regarding the manufacturing process of the additive, including details on the composition of the intermediate products were provided. With the new information on the manufacturing and on the test items used in the toxicological studies evaluated in 2013, the Panel identified the need for new genotoxicity studies, while confirmed the appropriateness of the test item used in the subchronic oral toxicity study. New genotoxicity studies were submitted by the applicant and the results of the tests showed no genotoxic potential of the test items. The Panel considered that the conclusions drawn in the subchronic oral toxicity study previously submitted can be considered valid; the results showed no evidence for adverse effects. Therefore, the Panel concluded that the additive is safe for the consumers when used as a feed additive.

Keywords: zootechnical additive, digestibility enhancer, endo-1,3(4)-β-glucanase, endo-1,4-β-xylanase, safety

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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 of Endofeed® DC (endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase) as a feed additive for chickens for fattening, laying hens, pigs for fattening and minor poultry and porcine species.

In 2013, the FEEDAP Panel adopted an opinion on the safety and efficacy of Endofeed® DC as a feed additive for chickens for fattening, laying hens, pigs for fattening and minor poultry and porcine species. The safety of the additive for the target species, user and environment and the efficacy were established at that time. In the previous assessment, the applicant provided in order to address the safety for the consumer a bacterial reverse mutation assay and an *in vitro* micronucleus test, and a subchronic oral toxicity study. However, considering the limitations on the description of the manufacturing process, the characterisation of the additive and on the toxicological studies provided the Panel could not conclude on the safety for the consumer. The applicant has now provided new data/information on the manufacturing process, characterisation of the additive and toxicological studies to assess the safety for the consumer, which are the main subject of this opinion.

In the current assessment, the applicant provided complete and detailed information regarding the manufacturing process of the additive, including details on the composition of the intermediate products. Moreover, the applicant provided supplementary information regarding the composition of the additive, the absence of the production strain, the content of mycotoxins as well as secondary metabolites of the production strain.

With the newly provided information on the manufacturing and on the test items used in the toxicological studies evaluated in 2013, the Panel identified the need for new genotoxicity studies while confirmed the appropriateness of the test item used in the subchronic oral toxicity study. New genotoxicity studies were submitted by the applicant. Due to the characteristics of the manufacturing process, the applicant prepared extracts of the fermentation product: water and dimethylsulfoxide extracts were prepared in order to ensure the recovery of any product present in the fermentation product. The two extracts obtained were tested separately in the bacterial reverse mutation test and also in the *in vitro* mammalian cell micronucleus test. The results of the tests showed no genotoxic potential of the test items. The Panel considered that the conclusions drawn in the subchronic oral toxicity study previously submitted can be considered valid; the results showed no evidence for adverse effects.

The results obtained in the genotoxicity studies and in the subchronic oral toxicity study did not indicate any reason for concern for consumer safety arising from the use of the product as a feed additive. Therefore, the Panel concluded that the additive is safe for the consumers when used as a feed additive.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003 establishes rules governing the Community authorisation of additives for use in animal nutrition and in particular, Article 9 thereof defines the terms of such authorisation by the Commission.

The applicant Andrés Pintaluba S.A. is seeking an authorisation of its endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase to be used as zootechnical additive (Table 1).

Table 1: Description of the substances

Category of additive	Zootechnical additives
Functional group of additive	Digestibility enhancers
Description	Endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase
Target animal category	Chickens for fattening, laying hens, pigs for fattening and minor poultry and porcine species
Applicant	Andrés Pintaluba S.A.
Type of request	Update opinion

On 11 July 2013, the Panel on Additives and Products or Substances used in Animal Feed of the Authority, in its opinion on the safety and efficacy of the product, could not conclude on the consumer safety based on the lack of adequate studies provided by the applicant.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the safety assessment and allow a revision of the Authority's opinion.

The data generated by the applicant and compiled in the above-mentioned supplementary information have been sent directly to the Authority by the applicant.

In view of the above, the Commission asks the Authority to deliver an updated opinion on the safety of endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase as zootechnical additive, based on the additional data submitted by the applicant.

1.2. Additional information

Endofeed® DC is an enzyme preparation that contains endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase. EFSA issued an opinion on the use of Endofeed® DC as a zootechnical additive (functional group: digestibility enhancers) for chickens for fattening, laying hens, pigs for fattening and minor poultry and porcine species (EFSA FEEDAP Panel, 2013). However, the manufacturing process was not fully described and the Panel could not conclude on the safety of the additive for the consumers.

2. Data and methodologies

2.1. Data

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

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of Endofeed® DC is in line with the principles laid down in Regulation (EC) No 429/2008[3] 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: Microbial Studies (EFSA, 2008), and Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b).

[1] FEED dossier reference: FAD-2014-0018.
[2] FEED dossier reference: FAD-2009-0015.
[3] 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.

3. Assessment

Endofeed® DC is an enzyme preparation which contains per gram, a minimum of 1,100 Units of endo-1,3(4)-β-glucanase (glucanase) and 1,600 Units of endo-1,4-β-xylanase (xylanase) that is intended to be used as a zootechnical additive (functional group: digestibility enhancers) for chickens for fattening, laying hens, pigs for fattening and minor poultry and porcine species. The enzymes are produced by *Aspergillus niger* NRRL 25541.

In the assessment performed in 2013 (EFSA FEEDAP Panel, 2013), the safety of this additive for the target species, user and environment and the efficacy were established. However, considering the limitations on the description of the manufacturing process, the characterisation of the additive and on the toxicological studies provided, the Panel could not conclude on the safety for the consumer. The applicant has now provided new data/information on the manufacturing process, characterisation of the additive and toxicological studies to assess the safety for the consumer, which is the main subject of this opinion.

3.1. Characterisation[4]

In the previous assessment (EFSA FEEDAP Panel, 2013), the information regarding the manufacturing missed relevant information for the fermentation steps and the characteristics of the resulting products, including the presence of the production strain.

The applicant submitted in the current assessment detailed information on the manufacturing process: including explanations of the different fermentation steps, on the resulting products (composition) and on the methodologies followed to kill the production strain. This information allowed the Panel to fully characterise the manufacturing and the composition of the additive itself.

The applicant accompanied the information on the manufacturing with data on the batch-to-batch variation in the enzyme activity, the absence of the production strain and purity data.

The batch-to-batch variation in five batches showed compliance with the minimum specifications (mean value of 1,388 glucanase U/g product (1,188–1,534 U/g) and 2,000 U xylanase/g (1,680–2,314 U/g)).[5] The presence of the production strain in the final product was evaluated in the same batches of the additive and was not found (limit of detection 5 colony forming units (CFU)/g).[6]

The raw material used to prepare the additive (four batches), the final additive (five batches) and a water extract of the additive (one batch) were analysed for the presence of mycotoxins in a multimycotoxin screening test.[7] The mycotoxins investigated were found to be below the limit of quantification.[8,9] One batch of the additive was analysed for secondary metabolites that can be produced by *A. niger* including malformin C (0.3 mg/kg), oxalic acid (26 g/kg), nigragillin (0.2 mg/kg), aurasperone (not detected, limit of detection < 500 μg/kg) and nigerazines (not detected, limit of detection < 100 μg/kg).[10]

New studies looking for the presence of antimicrobial activity were provided: the freeze-dried water extract of the additive (used in the genotoxicity studies, see below) showed no antimicrobial activity against five bacterial strains recommended by EFSA (EFSA, 2008b).[11] The applicant declared that no antimicrobial substances are used in the manufacturing process.[12]

[4] This section has been amended following the confidentiality claims made by the applicant.
[5] Technical dossier FAD-2014-0018/Annex II.3.4.
[6] Health Canada Method for the microbiological analysis of foods, yeast and moulds, method MFHPB-22.
[7] Technical dossier FAD-2014-0018/Supplementary information July 2015/Annexes II.1.4.3, II.1.4.4 and II.1.4.5.
[8] The mycotoxins investigated in the substrate and final product were: aflatoxins B1 (< 5 μg/kg), B2 (< 3 μg/kg), G1 (< 7 μg/kg) and G2 (< 9 μg/kg), deoxynivalenol (< 100 μg/kg), fumonisin B1 (< 40 μg/kg), B2 (< 100 μg/kg) and B3 (< 80 μg/kg), HT-2 toxin (< 80 μg/kg), ochratoxin A (< 8 μg/kg), sterigmatocystin (< 4 μg/kg), T-2 toxin (< 90 μg/kg) and zearalenone (< 70 μg/kg).
[9] The mycotoxins investigated in the water extract were: aflatoxins B1 (< 0.5 μg/kg), B2 (< 0.3 μg/kg), G1 (< 0.7 μg/kg) and G2 (< 0.9 μg/kg), deoxynivalenol (< 10 μg/kg), fumonisin B1 (< 4 μg/kg), B2 (< 10 μg/kg) and B3 (< 8 μg/kg), HT-2 toxin (< 8 μg/kg), ochratoxin A (< 0.8 μg/kg), sterigmatocystin (< 0.4 μg/kg), T-2 toxin (< 9 μg/kg), zearalenone (< 7 μg/kg), diacetoxyscirpenol (< 10 μg/kg), acetyldeoxinivalenol (< 13 μg/kg), ergocristine (< 5 μg/kg), ergocryptine (< 6 μg/kg), ergosine (< 9 μg/kg), neosolaniol (< 10 μg/kg), nivalenol (< 7 μg/kg), α-zearalenol (< 5 μg/kg) and β-zearalenol (< 7 μg/kg).
[10] Technical dossier/Supplementary information November 2016/Annex II.1.4.7.
[11] Technical dossier FAD-2014-0018/Annex II.1.4.6.
[12] Technical dossier FAD-2014-0018/Supplementary information November 2016.

3.2. Safety for the consumer[13]

In the previous assessment (EFSA FEEDAP Panel 2013), the applicant provided a bacterial reverse mutation assay and an *in vitro* micronucleus test, and a subchronic oral toxicity study. However, the Panel could not conclude on the suitability of the studies, due to the characteristics of the test items used, its relationship with the additive under assessment and methodologies followed.

With the information provided in the current assessment on the manufacturing and on the test items used in the studies evaluated in 2013, the Panel identified the need for new genotoxicity studies while confirmed the appropriateness of the test item used in the subchronic oral toxicity studies (see below). The applicant has provided new genotoxicity studies.

3.2.1. Genotoxicity studies

Owing to the characteristics of the manufacturing process and its resulting products, the applicant was requested to use extracts of the fermentation product. To ensure the complete extraction of possible by-products from the fermentation product, water and dimethylsulfoxide (DMSO) extracts were prepared. Aliquots of the fermentation product were separately suspended in water or in DMSO, overnight under continuous stirring. The samples obtained after separation of cells and particles, were concentrated by freeze-drying, in the case of the water extract or by vacuum evaporation at 50°C, in the case of the DMSO extract.

3.2.1.1. Bacterial reverse mutation tests

The two extracts were tested separately in *Salmonella* Typhimurium strains TA1535, TA1537, TA98, TA100, TA102 following the OECD Guideline 471.[14] The freeze-dried water extract was diluted in distilled water and tested up to a concentration of 5,000 μg/plate. The DMSO extract was diluted in DMSO and the maximum concentrations tested were 40 μg/plate or mL, due to a considerable precipitation that hindered the scoring, although no cytotoxicity was reported. For each extract, two independent experiments were conducted, with and without metabolic activation (S9 mix from rat livers induced by Aroclor 1254). The second assay was performed according to the pre-incubation method with metabolic activation and according to the plate incorporation without metabolic activation. With the DMSO extract and owing to a dosage error in the second experiment, a third experiment only with metabolic activation (pre-incubation method) was conducted.

With the water extract, neither cytotoxicity nor precipitation is observed. No increase in the number of revertants over the threshold of biological significance (twofold) and/or over historical control was observed in any experimental condition, while the positive controls produced the expected mutagenic effect.

With the DMSO extract in the third experiment, a 2.5 times increase in the number of revertants was noted at the highest dose of 30 μL/mL in strain TA98. This increase was not confirmed in a fourth experiment performed under the same experimental conditions. No other increase in the number of revertants was observed. The positive controls performed as expected.

3.2.1.2. *In vitro* micronucleus test

The two extracts were tested separately in the *in vitro* mammalian cell micronucleus test on TK6 lymphoblastoid human cells, following the OECD Guideline 487.[15] The freeze-dried water extract was diluted in distilled water and a top concentration of 2,000 μg/mL was selected on the basis of a preliminary cytotoxicity test. The DMSO extract was diluted in DMSO and the test concentrations were defined as percentages; the top concentration was 0.5%, based on a preliminary cytotoxicity test. The following treatment schedules were used: 3 h' treatment followed by 24 h' recovery time, with and without metabolic activation (S9 mix from rat livers induced by Aroclor 1254); 27 h continuous treatment without recovery time, only without metabolic activation. Two independent cultures were used and 1,000 cells per culture per concentration were scored.

The two extracts did not induce any statistically significant increase in the number of micronucleated cells at any of the concentrations analysed, while the positive controls were clearly effective, showing the sensitivity of the test system.

[13] This section has been amended following the confidentiality claims made by the applicant.
[14] Technical dossier FAD-2014-0018/Supplementary information July 2015/Annex III.2.2.5 and III.2.2.7.
[15] Technical dossier FAD-2014-0018/Supplementary information July 2015/Annexes III.2.2.6 and III.2.2.8.

3.2.2. Subchronic oral toxicity study

In the previous assessment, the applicant provided a subchronic oral toxicity study in rats (EFSA FEEDAP Panel, 2013). In that study, the rats were fed the additive instead of the fermentation product used to formulate the additive as recommended by the Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b). The supplementary information submitted in the current assessment allows the Panel to conclude that in this case the use of the additive is valid. In particular, considering the manufacturing process, resulting product and the highest treatment concentration (12.5 g/kg feed) the actual treatment dosage can be considered sufficient. The results showed that there is no evidence for adverse effects.

3.2.3. Conclusions on the safety for the consumer

The results obtained 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.

3.3. 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[16] and Good Manufacturing Practice.

4. Conclusion

The use of Endofeed® DC as a feed additive does not give rise to safety concerns for consumers.

Documentation provided to EFSA

1) Supplementary information Endofeed® DC. April 2014. Submitted by Andrés Pintaluba S.A.
2) Supplementary information Endofeed® DC. Supplementary information. July 2015. Submitted by Andrés Pintaluba S.A.
3) Supplementary information Endofeed® DC. Supplementary information. November 2016. Submitted by Andrés Pintaluba S.A.

References

EFSA (European Food Safety Authority), 2008. Technical Guidance: microbial studies. EFSA Journal 2008;6 (10):836, 3 pp. doi:10.2903/j.efsa.2008.836

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Technical guidance: tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. doi:10.2903/j.efsa.2011.2175

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 14 pp. doi:10.2903/j.efsa.2012.2536

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance for establishing the safety of additives for the consumer. EFSA Journal 2012;10(1):2537, 12 pp. doi:10.2903/j.efsa.2012.2537

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of Endofeed® DC (endo-1, 3(4)-beta-glucanase and endo-1, 4-beta-xylanase) as a feed additive for chickens for fattening, laying hens, pigs for fattening and minor poultry and porcine species.. EFSA Journal 2012;11(8):3322, 19 pp. doi:10.2903/j.efsa.2013.3322

Abbreviations

CFU	colony-forming unit
DMSO	dimethylsulfoxide
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed

[16] 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.