SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 16 May 2017

doi: 10.2903/j.efsa.2017.4856

Safety and efficacy of Amylofeed® (endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase and α-amylase) as a feed additive for piglets and minor porcine species

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Abstract

Amylofeed® is a preparation of endo-1,3(4)-β-glucanase, endo-1,4-β-xylanase and α-amylase aimed to be used as a feed additive for piglets and young minor porcine species. In a previous assessment, the safety of the additive for the target species, user and environment were established. However, the safety for the consumer and the efficacy of the product could not be established. In that previous assessment, the limitations on the description of the manufacturing process, the characterisation of the additive and on the toxicological studies provided did not allow the Panel to conclude on the safety for the consumer. The applicant has now provided new data/information to assess the safety for the consumer and also new studies in order to support the efficacy of the additive in the target species. The enzymes present in the product are obtained from two different strains and from two different fermentation processes. 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. New genotoxicity studies were submitted by the applicant and the results showed no genotoxic potential of the test items. The Panel considered that the conclusions drawn in the subchronic oral toxicity study performed and previously submitted were valid for the current assessment; the results showed no evidence for adverse effects. Consequently, the Panel concluded that the additive is safe for the consumers when used as a feed additive. For the evaluation of the efficacy of the additive, the Panel considered four long-term trials done in weaned piglets. Based on these data, the Panel concluded that the additive has a potential to be efficacious in weaned piglets at the nominal dose of 500 mg/kg feed and extrapolated this conclusion to growing minor porcine species.

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

Requestor: European Commission

Question number: EFSA-Q-2014-00503

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Suggested citation: 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, Brantom P, Dierick NA and Anguita M, 2017. Scientific Opinion on the safety and efficacy of Amylofeed® (endo-1,3(4)-β-glucanase and endo-1, 4-β-xylanase and α-amylase) as a feed additive for piglets and minor porcine species. EFSA Journal 2017;15(6):4856, 11 pp. https://doi.org/10.2903/j.efsa.2017.4856

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

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 Amylofeed® (endo-1,3(4)-β-glucanase, endo-1,4-β-xylanase and α-amylase) as a feed additive for piglets and young minor porcine species.

In 2013, the FEEDAP Panel adopted an opinion on the safety and efficacy of Amylofeed® (endo-1,3(4)-β-glucanase, endo-1,4-β-xylanase and α-amylase) as a feed additive for piglets and minor porcine species. The safety of the additive for the target species, user and environment was established at that time. However, the safety for the consumer and the efficacy of the product could not be established. The applicant has now provided new data/information and toxicological studies to assess the safety for the consumer. The applicant submitted also new studies in order to support the efficacy of the additive in the target species.

The enzymes present in the product are obtained from two different strains and from two different fermentation processes. In the current assessment, the applicant provided complete and detailed information regarding the manufacturing process of the additive. Moreover, the applicant provided supplementary information regarding the composition of the additive, the absence of the production strain in the final product and the content of mycotoxins as well as secondary metabolites of the production strains.

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. New genotoxicity studies were submitted by the applicant. Due to the characteristics of the manufacturing process, the applicant prepared extracts of the fermentation products: water and dimethylsulfoxide extracts were prepared in order to ensure the recovery of any product present in the fermentation products. The extracts obtained from each fermentation product 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 performed and previously submitted can be considered valid for the current assessment; the results showed no evidence of adverse effects.

For the evaluation of the efficacy of the additive, the Panel considered four long-term trials done in weaned piglets. The data from these four studies were pooled and statistically analysed. The data showed an improvement on the body weight and on the feed to gain ratio in the piglets fed with the additive at a nominal dose of 500 mg additive/kg feed. Therefore, the Panel concluded that the additive has a potential to be efficacious in weaned piglets at the nominal dose of 500 mg/kg feed and extrapolated this conclusion to growing minor porcine species. The analyses of the enzyme activity in the diets showed a higher glucanase and xylanase activities compared to the intended ones. Consequently, the Panel could not conclude on the efficacy at the recommended dose in terms of enzyme activity.

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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 EC 3.2.1.6, endo-1,4-β-xylanase EC.3.2.1.8 and α-amylase EC 3.2.1.1, 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 EC 3.2.1.6 Endo-1,4-β-xylanase EC 3.2.1.8 and α-amylase EC 3.2.1.1
Target animal category	Piglets (weaned) and young minor porcine species
Applicant	Andrés Pintaluba S.A.
Type of request	Update opinion

On 8th October 2013, the Authority, in its opinion on the safety and efficacy of the product, could not conclude on the consumer safety based on the toxicological studies provided and the lack of information on the manufacturing process that led to an insufficient characterisation of the product. In addition the efficacy of endo-1,3(4)-β-glucanase EC 3.2.1.6, endo-1,4-β-xylanase EC 3.2.1.8 and α-amylase EC 3.2.1.1 was not demonstrated from the data provided by the applicant.

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

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

In view of the above, the Commission asks the Authority to deliver an opinion on safety and efficacy of endo-1,3(4)-β-glucanase EC 3.2.1.6, endo-1,4-β-xylanase EC 3.2.1.8 and α-amylase EC 3.2.1.1 as zootechnical additive based on the additional data submitted by applicant.

1.2. Additional information

Amylofeed® is an enzyme preparation that contains endo-1,3(4)-β-glucanase, endo-1,4-β-xylanase and α-amylase. EFSA issued in 2013 an opinion on the use of Amylofeed® as a zootechnical additive (functional group: digestibility enhancers) for piglets and young minor porcine species (EFSA FEEDAP Panel, 2013). However, the FEEDAP Panel could not conclude on the safety of the additive for the consumers and the efficacy of the additive in weaned piglets was not demonstrated.

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 Amylofeed® 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,

[1] FEED dossier reference: FAD-2014-0019.
[2] FEED dossier reference: FAD-2010-0353.
[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.

2008), and Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b).

3. Assessment

Amylofeed® contains, per gram, a minimum activity of 275 Units (U)[4] of endo-1,3(4)-β-glucanase (glucanase; Enzyme Commission number (EC) 3.2.1.6), 400 U[5] of endo-1,4-β-xylanase (xylanase; EC 3.2.1.8) and 3,100 U[6] of α-amylase (amylase; EC 3.2.1.1). It is intended to be used as a zootechnical additive (functional group: digestibility enhancers) for weaned piglets and young minor porcine species at a recommended dose of 138 glucanase, 200 xylanase and 1,550 amylase U/kg complete feed (provided by 500 mg additive/kg feed).

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

3.1. Characterisation[7]

In the previous assessment (EFSA FEEDAP Panel, 2013), the dossier missed relevant information regarding the fermentation steps, the characteristics of the resulting products (including the composition and the presence of the production strains) and on the final composition of the additive. The applicant has now submitted new information.

Amylofeed® contains two fermentation products obtained in independent fermentations, one from *Aspergillus niger* NRRL 25541 and another one from *Aspergillus oryzae* ATTC 66222. The source of the xylanase and glucanase present in the additive is the fermentation product from *A. niger* and the source of amylase is the fermentation product from *A. oryzae*. The applicant submitted detailed information for each fermentation process and preparation of the additive including: the different fermentation steps, composition of the resulting products, the methodologies followed to kill the production strains, while preserving the xylanase, glucanase and amylase enzyme activities and on the composition of the additive. The information allowed the Panel to fully characterise the manufacturing process and the composition of the additive itself. The applicant accompanied the information with data on the batch to batch variation of the enzyme activity, absence of the production strains and purity data of the additive.

The batch to batch variation in five batches showed compliance with the minimum specifications (mean value of 584 glucanase U/g product (534–616 U/g), 523 U xylanase/g (480–577 U/g) and 5,206 U amylase/g (4,725–5,589 U/g)).[8] The presence of the production strains in the final product was evaluated in the same batches of the additive and was not found.

[4] 1 U is the amount of enzyme that liberates 1 micromole of reducing sugars (glucose equivalents) from oat beta-glucan per minute at pH 4.0 and 30°C.
[5] 1 U is the amount of enzyme that liberates 1 micromole of reducing sugars (glucose equivalents) from rye arabinoxylan per minute at pH 4.0 and 30°C.
[6] 1 U is the amount of enzyme that liberates 1 micromole of reducing sugars (glucose equivalents) from wheat starch per minute at pH 5.0 and 30°C.
[7] This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003
[8] Technical dossier FAD-2014-0019/Annex II.3.6.

The substrate for fermentation 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.[9] The mycotoxins investigated were found to be below the limit of quantification.[10,11] One batch of the additive was analysed for secondary metabolites produced by the two production strains. The following metabolites that can be produced by *A. niger* or by *A. oryzae* were investigated: malformin C (LOD = 0.1 mg/kg), oxalic acid (23 g/kg), nigragillin (0.2 mg/kg), aurasperone (not detected, LOD = 500 μg/kg), nigerazines (not detected, LOD = 100 μg/kg), cyclopiazonic acid (not detected, LOD = 100 μg/kg), 3-nitropropionic acid (not detected, LOD = 800 μg/kg), kojic acid (not detected, LOD = 600 μg/kg), maltoryzine (not detected, LOD not given), violacetin (not detected, LOD = 100 μg/kg) and aspergillomarasmine (not detected, LOD not given).[12]

New studies looking for the presence of antimicrobial activity were provided: the freeze-dried water extracts of the fermentation products (used in the genotoxicity studies, see below) showed no antimicrobial activity against five bacterial strains recommended by EFSA (2008).[13] The applicant declared that no antimicrobial substances are used in the manufacturing process.

3.2. Safety for the consumer[7]

In the previous assessment (EFSA FEEDAP Panel, 2013), the applicant provided a bacterial reverse mutation assay, an *in vitro* micronucleus test and a subchronic oral toxicity study in rat. However, the Panel could not conclude on the suitability of the studies, due to the characteristics of the test items used, the unclear relationship with the additive under assessment and the 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 subchronic oral toxicity studies previously evaluated (see below). The applicant has provided new genotoxicity studies.

3.2.1. Genotoxicity studies

Owing to the characteristics of the fermentation processes and the resulting products, the applicant was requested to use extracts of each fermentation product to perform the new genotoxicity tests. To ensure the complete extraction of possible by-products from the fermentation products, water and dimethylsulfoxide (DMSO) extracts were prepared. Aliquots of the fermentation products were separately suspended in water or in DMSO, kept under continuous stirring overnight. 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

Fermentation product of Aspergillus niger NRRL 25541

The two extracts were tested separately in *Salmonella* Typhimurium strains TA1535, TA1537, TA98, TA100 and 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

[9] Technical dossier FAD-2014-0019/Supplementary information December 2015/Annexes II.1.4.1, II.1.4.2 and II.1.4.3.

[10] 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), T-2 toxin (< 90 μg/kg), HT-2 toxin (< 80 μg/kg), ochratoxin A (< 8 μg/kg), sterigmatocystin (< 4 μg/kg) and zearalenone (< 70 μg/kg).

[11] 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), T-2 toxin (< 9 μg/kg), HT-2 toxin (< 8 μg/kg), ochratoxin A (< 0.8 μg/kg), sterigmatocystin (< 0.4 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), alpha-zearalenol (< 5 μg/kg) and beta-zearalenol (< 7 μg/kg).

[12] Technical dossier FAD-2014-0019/Supplementary information November 2016/Annex II.1.4.5.

[13] Technical dossier FAD-2014-0019/Supplementary information December 2015/Annex II.1.4.4.

[14] Technical dossier FAD-2014-0019/Supplementary information December 2015/Annex III.2.2.2.6.1 and III.2.2.2.8.1.

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 was observed. No increase in the number of revertants over the threshold of biological significance (two fold) 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.

Fermentation product Aspergillus oryzae ATTC 66222

The two extracts were tested separately in *Salmonella* Typhimurium strains TA1535, TA1537, TA98, TA100 and TA102 following the OECD Guideline 471.[15] 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 highly viscous and it was diluted in DMSO and the maximum concentrations tested were 10 μL/plate or mL. 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.

In the water extract, neither cytotoxicity nor precipitation was observed, but some opacity, attributed to the presence of histidine in the test item, was reported at the top concentration. Statistically significant increases in the number of revertant colonies were noted in strain TA102 without metabolic activation, and in strains TA1535 and TA100 with metabolic activation with pre-incubation. However, these increases were attributed to the presence of traces of histidine in the test item. In fact, no such increase was reported when the treat and wash method, that allows the elimination of peptides and amino acids from the medium, was applied. In the DMSO extract, under the experimental conditions applied, no mutagenic activity was revealed.

3.2.1.2. *In vitro* micronucleus test

Fermentation product Aspergillus niger NRRL 25541

The two extracts were tested separately in the *in vitro* mammalian cell micronucleus test on TK6 lymphoblastoid human cells, following the OECD Guideline 487.[16] 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 hours 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.

Fermentation product Aspergillus oryzae ATTC 66222

The two extracts were tested separately in the *in vitro* mammalian cell micronucleus test on TK6 lymphoblastoid human cells, following the OECD Guideline 487.[17] The freeze-dried water extract was diluted in distilled water and the DMSO extract was diluted in DMSO. The top concentration tested was limited by the cytotoxicity of the test item. The treatment schedules followed OECD guideline recommendation as reported above and 1,000 cells per concentration per two independent culture were scored. The two extracts showed negative results, while the positive controls performed as expected.

[15] Technical dossier FAD-2014-0019/Supplementary information December 2015/Annex III.2.2.2.6.2 and III.2.2.2.8.2.
[16] Technical dossier FAD-2014-0019/Supplementary information December 2015/Annexes III.2.2.2.7.1 and III.2.2.2.9.1.
[17] Technical dossier FAD-2014-00189/Supplementary information December 2015/Annexes III.2.2.2.7.2 and III.2.2.2.9.2.

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, Amylofeed®, instead of the fermentation products used to formulate the additive as recommended by the Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b). However, with the limited information previously submitted, the Panel could not conclude on the suitability of the test item. 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 the actual treatment dosage for the two fermentation products 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 studies considered do not indicate any reason for concern for consumer safety arising from the use of the product as a feed additive.

3.3. Efficacy in the target species

The additive is to be used in feed for piglets and young minor porcine species at a recommended dose of 138 glucanase, 200 xylanase and 1,550 amylase U/kg complete feed (provided by 500 mg additive/kg feed).

In 2013, the Panel evaluated five trials in weaned piglets (EFSA FEEDAP Panel, 2013). Four of them were rejected due to the use of medication and/or due to the short duration of the studies. One of the studies was retained as acceptable and has been also included in the current assessment (trial 1 below). A total of five trials were assessed in the current assessment, four of which were newly submitted. One of these trials was not considered due to the limitations found in the study design; the diets containing the additive were different in terms of composition to the one in the control diet, not representing therefore a suitable control.[18] A summary of the trial design is presented in Table 2 and the results are presented in Table 3. In the four studies, weaned piglets were used and the experimental groups considered a control diet and at least a diet with the recommended dose of the additive. The diets were analysed for enzyme activities and the results are reported in Table 3. Before the start of the study, the piglets in trials 2–4 were allowed for a short period (from 2 to 6 days) of acclimatisation to the rooms and diets. The duration of all the studies was of 42 days. Health was monitored throughout the duration of the studies. Feed intake and body weight were measured and feed to gain were calculated. In trial 4, a digestibility study was conducted but the data is not reported because the effect on the metabolisable energy content was not evaluated. In each individual study, the data was analysed by an ANOVA, using the pen as the experimental unit and considering the effect of the sex where relevant.

[18] Technical dossier FAD-2014-0019/Supplementary information December 2015/Annex IV.3.6.

Table 2: Trial design and dosages of the efficacy trials performed in weaned piglets

Trial	Total no animals (animals/replicate) Replicates/group	Breed Sex Initial BW	Diet composition (form)	Enzyme activity (U/kg) Glucanase/ xylanase/amylase	
				Intended	Analysed
1[(a)]	640 (10) 16	Large White × Landrace ♀,♂ mixed 50:50 7.7 kg	Barley, wheat, maize, soya bean meal (pellets)	0/0/0 110/160/1,240 138/200/1,550 165/240/1,860	0/0/0 116/105/744 281/160/1,091 199/178/1,337
2[(b)]	144 (4) 18	Goland × Duroc BM ♀,♂castrated, sex separated 7.5 kg	Maize, soya bean meal (mash)	0/0/0 138/200/1,550	0/0/0 620/562/1,872
3[(c)]	144 (4) 18	Bompieri × Goland ♀,♂castrated, sex separated 7.1 kg	Maize, soya bean meal (mash)	0/0/0 138/200/1,550	7/84/0 378/507/1,056
4[(d)]	144 (6) 12	Commercial breed ♀,♂ mixed 7.9 kg	Barley, wheat, soya bean meal (mash)	0/0/0 138/200/1,550	0/0/0 338/719/1,542

(a): Technical dossier FAD-2014-0019/Annex IV.3.1.
(b): Technical dossier FAD-2014-0019/Supplementary information December 2015/Annex IV.3.7.
(c): Technical dossier FAD-2014-0019/Supplementary information November 2016/Annex IV.3.8.
(d): Technical dossier FAD-2014-0019/Supplementary information January 2017/Annex IV.3.9.

Table 3: Effects of Amylofeed® on the performance of weaned piglets

Trial	Enzyme activity (U/kg)	Daily feed intake (kg)	Body weight (kg)		Feed to gain ratio	Dead (n)
			Initial	Final		
1	0/0/0	0.676	7.8	25.4[a]	1.61	2
	110/160/1,240	0.689	7.8	26.0[a,b]	1.59	3
	138/200/1,550	0.685	7.8	26.4[a]	1.55	0
	165/240/1,860	0.678	7.8	25.5[b]	1.61	3
2	0/0/0	0.850	7.6	27.8[b]	1.76[a]	0
	138/200/1,550	0.840	7.4	28.9[a]	1.65[b]	0
3	0/0/0	0.760	7.0	25.9	1.69	0
	138/200/1,550	0.772	7.2	26.4	1.69	0
4	0/0/0	0.606	7.9	25.9	1.42	2
	138/200/1,550	0.620	7.9	26.2	1.42	1
Pooled data	0/0/0	0.736	7.5	26.3[b]	1.64[a]	–
	138/200/1,550	0.740	7.5	27.1[a]	1.59[b]	–

[a,b]: Values within one column for the same study with different superscripts are different ($p < 0.05$).

The results of the individual studies showed a significant improvement on the final body weight in two trials (trials 1 and 2) and on the feed to gain ratio in one trial (trial 2) at the recommended dose (nominal basis). In trial 3, there was an interaction between the treatment and the sex; significant improvement on the body weight of the piglets at the recommended dose (nominal basis) was seen only in males.

The applicant pooled the data of the four studies considering the control groups and the group with the recommended dose.[19] The effects of the treatment and of the study were included in the model as well as their interaction. No interactions between treatment and study were found. The results showed a higher final body weight (27.1 vs 26.3 kg, $p < 0.05$) and a better feed to gain ratio (1.59 vs 1.64, $p < 0.05$) in the group receiving the recommended dose. The Panel notes that analytical results of the enzyme activity in the diets showed a large overage in glucanase and xylanase in trials 2, 3 and 4. The mean values for glucanase and xylanase in those trials were 445 and 596 U/kg feed, respectively, whereas the intended doses are 138 and 200 U/kg feed for glucanase and xylanase, respectively.

[19] Technical dossier FAD-2014-0019/Supplementary information January 2017/Annex IV.3.10.

The Panel concludes based on the results from the pooling study that the additive has a potential to be efficacious in weaned piglets at the nominal dose of 500 mg additive/kg feed. The mode of action is well known and it is assumed to be similar among porcine species, consequently, this conclusion can be extrapolated to growing minor porcine species. However, considering the analysed enzyme activities in feed, the Panel is not in a position to conclude on the effective doses in terms of enzyme activities.

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[20] and Good Manufacturing Practice.

4. Conclusions

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

Amylofeed® has a potential to be efficacious as a zootechnical additive in weaned piglets and growing minor porcine species at a nominal dose of 500 mg additive/kg feed. However, considering the analysed enzyme activities in feed, the Panel is not in a position to conclude on the effective doses in terms of enzyme activities.

Documentation provided to EFSA

1) Supplementary information Amylofeed®. May 2014. Submitted by Andrés Pintaluba S.A.
2) Supplementary information Amylofeed®. Supplementary information. December 2015. Submitted by Andrés Pintaluba S.A.
3) Supplementary information Amylofeed®. Supplementary information. November 2016. Submitted by Andrés Pintaluba S.A.
4) Supplementary information Amylofeed®. Supplementary information. January 2017. Submitted by Andrés Pintaluba S.A.

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of Amylofeed® (endo-1,3(4)-beta-glucanase, endo-1,4-beta-xylanase and alpha-amylase) as a feed additive for piglets and young minor porcine species. EFSA Journal 2013;11 (10):3430, 15 pp. https://doi.org/10.2903/j.efsa.2013.3430

Abbreviations

CFU	colony forming unit
DMSO	dimethylsulfoxide
EC	Enzyme Commission number
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
U	Unit

[20] 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.