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Safety and efficacy of a feed additive consisting of pancreatin from porcine pancreas (Pan-zoot) for dogs (Almapharm GmbH + Co KG)

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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 a pancreatic extract (Pan-zoot) as a zootechnical additive for dogs. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) could not conclude on the safety of Pan-Zoot as a feed additive for dogs under the proposed conditions of use. The FEEDAP Panel could not conclude on the skin/eye irritancy potential of the additive or on the dermal sensitisation potential. Owing to its proteinaceous nature, the additive is considered a respiratory sensitiser. The additive may induce allergic reactions to the exposed users. The Panel concluded that there is no need for an environmental risk assessment. The FEEDAP Panel could not conclude on the efficacy of the product as a feed additive at the recommended conditions of use.

Keywords: zootechnical additives, digestibility enhancers, pancreas extract, safety, efficacy, dogs

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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 Almapharm GmbH + Co KG[2] for the authorisation of the additive consisting of pancreatin from porcine pancreas glands (Pan-zoot) when used as a feed additive for dogs (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 29 October 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 pancreatin from porcine pancreas glands (Pan-zoot) when used under the proposed conditions of use (see **Section 3.1.4**).

1.2. Additional information

The additive contains pancreatin from porcine pancreas glands and is not authorised as a feed additive in the European Union.

Pancreatin (Chemical Abstracts Service (CAS) number 8049-47-6) contains various enzymes having proteolytic, lipolytic and amylolytic activity. Pancreas powder is described in the European Pharmacopoeia (monograph 01/2022:0350) with the following composition and purity specifications: one mg of pancreas powder should contain not less than 1.0 Ph Eur U of total proteolytic activity, 15 Ph Eur U of lipolytic activity and 12 Ph Eur U of amylolytic activity; it should contain a maximum of 5% fat and show a maximum of 5% loss on drying; microbial contamination is set to be $< 10^4$ colony forming unit (CFU)/g product for total aerobic microbial counts, $< 10^2$ CFU/g product for total yeasts and moulds counts, absence of *E. coli* and *Salmonella* in 10 g of product.

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[6] in support of the authorisation request for the use of pancreatin from porcine pancreas glands (Pan-zoot) as a feed additive. The dossier was received on 25/3/2021 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00464.

The FEEDAP Panel used the data provided by the applicant together with data from other sources.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substances in animal feed.[7] The Panel notes that during the risk assessment phase, the applicant provided some clarifications regarding the specifications and

[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] Almapharm GmbH + Co. KG, salzstraß 27, 87,499 Wildpoldsried im Allgäu, Germany.
[3] One unit of protease activity corresponds to the amount of enzyme that liberates per minute peptides with an absorption at 275 nm that is equivalent to the one of 1 micromole tyrosine.
[4] One unit of lipase activity corresponds to the amount of enzyme that liberates under the conditions of the assay of pH 9.0 and 37 °C per minute one microequivalent of acid.
[5] One unit of amylase activity corresponds to the amount of enzyme that splits starch under the conditions of the assay with an initial rate such that one microequivalent of glycosidic linkage is hydrolysed per minute.
[6] FEED dossier reference: FAD-2021-0076.
[7] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en

composition of the additive, and the conditions of use which were not available to the JRC at the time of the completion of the method.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of pancreatin from porcine pancreas glands (Pan-zoot) is in line with the principles laid down in Regulation (EC) No 429/2008[8] 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, 2017a), Guidance on the identity, characterisation, and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The product under assessment contains pancreatin from porcine pancreas glands and herein will be referred to as Pan-zoot. It is aimed to be used as a zootechnical feed additive (functional group: digestibility enhancer) in dogs.

3.1. Characterisation

3.1.1. Origin and extraction

[redacted][9]

[redacted][10]

[redacted]

3.1.2. Characterisation

The additive under assessment consists only of pancreatin of porcine pancreatic glands and it is specified for a loss on drying < 5% (at 60°), proteolytic activity > 3.5 Ph Eur U/mg, amylolytic activity of > 48 Ph Eur U/mg, and lipolytic activity > 55 Ph Eur U/mg.[11]

The batch-to-batch variation (analysed in 14 batches)[12] showed a water content of 2.9% (ranging 2.6–3.7%), proteolytic activity of 3.9 Ph Eur U^3/mg (ranging 3.2–4.7 Ph Eur U/mg), amylolytic activity 50.7 Ph Eur U^5/mg (ranging 43.2–57.5 Ph Eur U/mg) and lipolytic activity 57.4 Ph Eur U^4/mg (ranging 45.7–66.3 Ph Eur U/mg). It is noted that three batches were not compliant with the specifications of the additive in terms of enzyme activity. Data was also made available for the content of fat (four batches below 2.5%) and total purines content (3 batches, ranging between 12.6 and 23.5 g/kg).[13]

At least three batches of the additive were analysed for chemical and microbial contamination[14] and included the analysis of arsenic (≤ 0.024 mg/kg), cadmium (≤ 0.096 mg/kg), lead (≤ 0.075 mg/kg),

[8] 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.
[9] Technical dossier/Section II/Annex II.06 and Supplementary information June 2022/Annexes/Sin_June_22_Annexes 1, 3 and 14.
[10] Technical dossier Section II/Annex II.03 and Supplementary information June 2022/Annex 3.
[11] Technical dossier/Section II/table 2.
[12] Technical dossier/Section II/Annex 02 and Supplementary information June 2022/Annexes 06, 08 and 23.
[13] Technical dossier/Supplementary information November 2022/ 2022-11-02 FAD-2021-0076 EFSA_letter SIn Response.
[14] Technical dossier/Section II/Annex 02 and 15 and Supplementary information June 2022/Annex 05.

mercury (< 0.02 mg/kg), cobalt (≤ 0.047 mg/kg), vanadium (< 0.2 mg/kg) and nickel (< 0.2 mg/kg), total aerobic microbial counts (≤ 4,700 CFU/g), total yeast and moulds (≤ 1,100 CFU/g), *E. coli* (not detected in 1 g), Enterobacteriaceae (< 10 CFU/g), bile-tolerant gram-negative bacteria (< 10 CFU/g), *Staphylococcus aureus* (not detected in 10 g or per g, depending on the method used), *Salmonella spp*. (not detected in 10 or 25 g).

[redacted][15]

The applicant investigated [redacted] inactivate potentially hazardous viruses derived from the raw material from which the feed additive is obtained.[16] [redacted] effective in inactivating the [redacted] viruses tested. In addition, five batches of the feed additive were tested for the presence of hepatitis E virus (in 100 g)[17] and one batch for the presence of Aujeszky virus,[18] and the viruses were not detected in any of the batches tested.

The product is presented in powder form and it has a bulk density between 500 and 700 kg/m^3. Data on the dusting potential was provided for three batches and showed values from 4.4 to 7.4 g/m^3.[19] The particle size distribution was measured in the same three batches and showed that 75.5% of the particles are < 100 μm, 57% are < 50 μm, 27% are < 10 μm and 3.5% < 1 μm.[20]

3.1.3. Stability and homogeneity

The stability of three batches of pancreas powder was measured when stored at 25°C for 3 years or at 40°C for 6 months.[21] Proteolytic, amylolytic and lipolytic activities were measured and showed losses between 3.2 and 3.7% for proteolytic, 2.8 and 3.2% for amylolytic and 3.4 and 3.6% for lipolytic when stored at 25°C. The batches stored at 40°C for 6 months showed losses below or equal to 1.5% for all enzyme activities.

The additive is to be used in complementary feeds. A so-called 'complementary feed' (three batches, prepared with the same batch of the additive) was manufactured, using 20% of additive (one batch) mixed with dextrose (50%), lactose (10%) and maltodextrin (20%).[22] The complementary feed was then stored for 13 weeks at room temperature (19–23°C), and the loss of enzyme activity was determined. The results showed negligible losses for lipolytic and amylolytic activity and an 8% loss for proteolytic activity.

Ten subsamples of the complementary feed were analysed for the amylase activity; the coefficient of variation was below 2%.

3.1.4. Conditions of use

The additive is to be used as a zootechnical additive in feed for dogs. The additive is to be added by means of a complementary feed which contains the additive in the range of 10–40%, and the remainder being feed materials such as lactose, dextrose, maltodextrin that allow easy mixing of the complementary feed into the dog wet feed. The amount of additive to be added equals 35 mg/g of crude fat in the feed (equiv. to 2,000 lipolytic Ph Eur U/g of crude fat).[23] According to the applicant this would correspond to 2 g additive/kg wet feed with a dry matter content of 24% and a crude fat content of up to 5.5%. The level should not exceed 7.7 g additive/kg complete feed (88% dry matter).

[15] Technical dossier/Section II/Annex II.02 and Supplementary information June 2022/Annex 23.
[16] Technical dossier/Section II/Annex_II_12.
[17] Technical dossier/Supplementary information June 2022/Annexes/SIn_June_22_Annex_07.
[18] Technical dossier/Section II/Annex_II_13.
[19] Technical dossier/Section II/Annex_II_04.
[20] Technical dossier/Section II/Annex_II_04 and Annex_II_05.
[21] Technical dossier/Section II/Annex_II_06.
[22] Technical dossier/Section II/Annex_II_07 and Annex_II_14.
[23] Technical dossier/Section II and Supplementary information June 2022/Annex_09.

Considering that the addition of the additive is at most 7.7 g/kg complete feed as is (with 88% dry matter), the amount expressed per dry matter feed would be 8.75 g additive/kg DM feed. A dog with a body weight of 15 kg may eat 250 g of feed dry matter per day (EFSA FEEDAP Panel, 2017c); the total maximum amount of additive received per dog and day would be 2.19 g (corresponding to at least 7,656 proteolytic, 105,000 amylolytic and 120,312 lipolytic Ph Eur U per dog and day) or 146 mg additive/kg body weight and day.

3.2. Safety

3.2.1. Safety of the additive for the target species

The additive is obtained by extraction from porcine pancreas, edible offal according to Regulation (EC) No 853/2004, and the active substances present in the product are enzymes. The safety assessment should consider also any hazard introduced by the extraction process. The genotoxic potential and toxicological profile of the additive as well as the additive's tolerance should be evaluated to establish the safety for the target species.

To evaluate the safety of the additive for the target species the FEEDAP Panel considered the composition of the additive, the literature/published references submitted by the applicant, and a tolerance trial conducted in dogs.

3.2.1.1. Toxicological profile

No toxicological data were submitted for the additive under assessment.

The pancreatic tissue and its components, per se, are not expected to have genotoxic potential. However, the extraction process to which the porcine pancreas is subjected may introduce hazards.

[redacted]

The applicant referred to the evaluation by the European Medicine Agency (EMA) on a no-longer authorised pharmaceutical product in EU which contains pancreatin,[26] indicating that it has a well-established human safety profile. In the report, there are available references to studies on pharmacodynamics/kinetics and toxicological studies.

Among the toxicological studies mentioned in EMA's report, the FEEDAP Panel considers that the studies conducted and reported by Saruc et al. (2012)[27] may be relevant for the current assessment. In that publication, the authors reported studies testing porcine lyophilized pancreas containing 30–80 US Pharmacopeial (USP) units of proteolytic activity per milligram and 15–40 USP units of lipolytic activity per milligram. The studies reported were all in hamsters, namely: two acute oral toxicity studies, a 15-day repeated oral toxicity study, and a 65-day repeated oral toxicity study. No relevant effects were observed for the acute oral toxicity studies, and the 15-day repeated oral toxicity study. However, in the 65-day oral toxicity study, the results showed a significantly lower body weight of the hamsters receiving the product at 400 mg/kg body weight (bw) per day compared to the control group, even though the feed and water intake was the same between the groups. The treated hamsters showed, compared to the untreated controls, significantly lower plasma levels of insulin, amylase, and lipase, while glucagon was higher. Moreover, the size of the pancreatic islets in the treated group was significantly smaller with a lesser number of insulin cells/islets; no differences were observed in the number of glucagon cells. In this study, the product was administered via water at 400 mg/kg bw and day, which would correspond to 6,000–16,000 USP lipolytic Units per kg body weight and day (USP lipolytic units equal EU Ph U).[28] The product used in the publication contained 15–40 lipase units per mg; the additive under assessment contains at least 55 lipase EU Ph units

[redacted]

[26] Technical dossier/Section III/Annex III.04.
[27] Technical dossier/Section III/Annex III.06.
[28] Technical dossier/Supplementary information June 2022/Annex 04.

per mg. Therefore, to obtain the same level of enzyme activity the dosage of the product under assessment could be from 110 to 290 mg additive under assessment/kg bw per day.[29] With the conditions of use established, the dogs may receive 150 mg additive under assessment/kg bw per day (see Section 3.1.4).

The EMA's report also mentions some safety concerns regarding the use of the product in humans including anaphylactic reactions; fibrosing colonopathy with a long-term administration of high doses; transmission of viral diseases; hyperuricaemia and hyperuricosuria (possibly due to the occurrence of components such as purine bases). The document also mentions the ulceration in the oral mucosa of dogs receiving powder pancreatic extracts (Rutz et al., 2002; Snead, 2006).

3.2.1.2. Tolerance trial in dogs

Eighteen adult dogs, nine Beagle dogs (body weight 10–15.5 kg) and nine Foxhound crossbreed dogs (body weight 22.5–30 kg), were randomly allocated to three experimental groups (representing 6 dogs per group, three of each breed).[30] According to the applicant, the dogs used for the tolerance study were clinically healthy animals and not showing exocrine pancreatic insufficiency based on the specific pancreatic lipase immunoreactivity (cPLI) in the serum on day 0 (all values < 55 μg/L).[31] The animals received a canned commercial (for mature dogs) wet feed (24% dry matter content; 4.3 MJ/kg, 90 g/kg crude protein and 55 g/kg crude fat); and the amount offered was estimated for each individual based on previous consumption data to keep the body weight constant. The analytical confirmation of the composition of the diet was not submitted by the applicant. The groups were a control group which received a placebo (greaves), a group that received the additive at the maximum recommended level (1×) of 2,000 lipolytic Ph Eur U/g crude fat and a group that received the additive at 6,000 lipolytic Ph Eur U/g crude fat representing 3× the maximum recommended level. The placebo and the additive were mixed with the commercial feed with 2 tablespoons of yoghurt (10% DM; 0.1% fat). The enzyme activities in the additive used were analytically confirmed. The calculated levels of additive received by the dogs would equate to 7.7 g additive/kg complete feed (with 88% DM) in the 1× group and 22.8 g/kg for the 3× group. The dogs received the placebo or the additive for 28 days and the daily food was divided into two meals (morning and afternoon). In each meal, the animals had 30 min access to food, after which any leftover was recorded.

The study was conducted on a blind basis. The general health and behaviour of the dogs were monitored daily, and a physical examination was performed once per week, including the week prior to the study. Every other day and following the morning feeding, the mucous membranes of the mouth and anus were checked for reddening, lesions, and other possible irritations or alterations. The intensity of reddening of the oral mucosa was graded on a three-grade scale (from light red, red, and dark red). The monitoring of the mucosae was extended for another 2 weeks following the end of the administration period. Animals were weighed on a weekly basis. On days 1, 14, and 28 of the trial, blood was sampled (fasted animals) and faecal samples were collected. The blood samples were analysed for haematological[32] and biochemical[33] parameters and the faecal samples were monitored for pH and scored according to its consistency. The data were analysed by a Kruskal-Wallis rank based nonparametric test and the significance level was set at $p \leq 0.05$.

No statistical differences ($p = 0.65$) were observed in average feed intake, which amounted to 1,325, 1,601 and 1,566 g wet feed per animal and day for control, 1× and 3× groups, respectively. The amount of additive ingested was 0, 3.23 and 9.30 g per animal and day for control, 1×, and 3× respectively. The average final body weight of the animals was not different ($p = 0.42$) between groups (19.3, 21.3, and 21.0 kg for control, 1×, and 3×), and no differences between initial and final body weight were identified within groups.

Faecal score and pH showed no differences among groups. Statistically significant differences were observed on creatinine kinase at day 28 (37, 47 and 40 U/L, control and 1× group were different) and

[29] The calculations using the proteolytic activity results in dosages ranging 54–146 mg/kg bw day.
[30] Technical dossier/Section III/Annex III.1 and Supplementary information June 2022 and November 2022.
[31] Technical dossier/Supplementary information November 2022.
[32] Including: total leucocytes, lymphocytes, monocytes, eosinophile granulocytes, basophile granulocytes, neutrophile granulocytes, erythrocytes, haematocrit and haemoglobin.
[33] Including: cholesterol, bilirubin, triglycerides, albumin and globulin (an quotient), total proteins, glucose, fructosamine, alpha-amylase, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, citokinase, gamma-glutamil transferase, glutamate dehydrogenase, blood urea nitrogen, creatinine, DGGR-lipase activity, specific pancreatic lipase immunoreactivity (cPLI), serum amyloid A, C-reactive protein, calcium, phopshporus, sodium, magnesium, potassium and iron.

albumin at day 1 (38, 37 and 40, 1× and 3× groups were different). A few haematology and blood chemistry parameters were out of the reference range.[34]

After 2 weeks under study, five animals (one in the placebo and four in the 3× group) displayed alterations in the oral mucosa for more than two consecutive checks. Three of the dogs receiving the 3× group showed the highest grading for reddening intensity in at least one check. In three of the 3× group, the reddening was still present for 7 days after the feeding of the product had been stopped, but only in one of them the severity of the lesion did not decrease with time. The dogs in the 3× group, displaying the highest graded reddening in the oral mucosa, were the ones that received the highest total amount of the additive (highest body weight). The serum concentration of C-reactive protein (cRP reference value < 15 mg/L) showed no significant increase in the exposed groups from day 1 to days 14 and 28.

Conclusions from the tolerance trial

The FEEDAP Panel notes that the study was done with a small number of animals; the analytical confirmation of the composition of the diets was not submitted, therefore, some of the exposure estimates are based on assumed values; and the pancreatic function of the animals, at the beginning and at the end, was studied but in a limited way (e.g. insulin not measured, trypsin-like immunoreactivity test not done).

The tolerance study did not show major adverse effects when feeding the dogs up to three times the recommended level, except for the presence of oral mucosal reddening in half of the dogs that received the 3× level. The animals affected were the heaviest ones receiving the higher amounts of the additive (on daily basis).

3.2.1.3. Synopsis

A study conducted in hamsters receiving for 65 days a pancreatin product at levels of pancreatin that would equate to the amounts the dogs would receive following the conditions of use, showed a significant reduction of plasma levels of insulin, amylase, and lipase in the treated animals compared to the controls. Moreover, it was also observed a significant reduction in the size of the pancreatic islets in the treated animals, with a smaller number of insulin cells/islets. These results suggest a down-regulation of the pancreatic function after the administration of pancreatic enzymes to the animals. The FEEDAP Panel considers that the effects shown in hamsters after 65-day administration of pancreatin are relevant for the safety evaluation of healthy dogs fed pancreatic enzymes for long-term periods. Consequently, it is considered that the use of the additive under assessment in the feed for healthy dogs at the recommended levels may result in a downregulation of the pancreatic function (endocrine/exocrine) over time.

The tolerance trial conducted with 6 dogs per group showed no major modifications in the parameters measured except for the appearance of oral mucosal reddening in the animals receiving the highest level of the additive. Reddening could be an allergic symptom. It is not clear if and to what extent other mucosal surfaces might be affected. The parameters measured, despite being relevant for the assessment of the tolerance, did not sufficiently address the potential effects of the additive, and its withdrawal, on the exocrine and endocrine pancreatic function of the dogs. In this regard, canine-specific pancreatic lipase immunoreactivity and cRP were not influenced by the additive; but canine trypsin-like immunoreactivity, one of the most sensitive and specific tests for detecting exocrine pancreatic insufficiency, and plasma insulin were not measured. The dogs received the test item for 28 days, in line with the requirements established for a tolerance trial in the guidance on the safety for the target species (EFSA FEEDAP Panel, 2017c). Therefore, the trial does not allow to study of the effect of a long-term administration and/or the impact of a withdrawal of the additive. A down-regulation of the pancreatic function in dogs by a long-term application of the additive cannot be excluded.

The Panel considers that the tolerance trial conducted in dogs and the data made available do not allow to exclude potential adverse effects of long-term supplementation of pancreatic enzymes on the pancreatic function in healthy dogs.

[34] The acute phase protein serum amyloid A levels were not different among groups, but above the reference value (>4.6 μg/L) in all three groups for unknown reasons. The report indicated that some haematology values were out of the normal reference ranges, namely: lymphocytes (day 1, 14 and 28) for all three groups, monocytes (day 1 and 14 for all three groups), eosinophil granulocytes for all three groups (on day 1) and in other time points depending on the group; glucose on day 28 in the control group; and iron on day 14 and 28 in the 3× group.

3.2.1.4. Conclusions

The data made available do not allow to exclude down-regulation of exocrine and endocrine pancreas functions and potential allergic effects mainly on mucosal surfaces. For an additive, of which the active substances may interact with endogenous substances, 28-day data would not provide sufficient assurance to conclude on the effects of a long-term administration.

The FEEDAP Panel cannot conclude on the safety of Pan-zoot as an additive to feed for dogs under the proposed conditions of use.

3.2.2. Safety for the users

No data was provided with the additive under assessment. Therefore, the Panel cannot conclude on the skin/eye irritancy potential of the additive or on the dermal sensitisation potential. Owing to its proteinaceous nature, the additive is considered a respiratory sensitiser. The additive may induce allergic reactions, as reported by Rutz et al. (2002).

3.2.3. Safety for the environment

The additive under assessment is intended to be used in dogs only. No environmental risk assessment is necessary for such use (EFSA FEEDAP Panel, 2019).

3.3. Efficacy

3.3.1. *In vitro* studies

In support of the efficacy of the additive, the applicant submitted three *in vitro* studies aiming to study the pre-caecal digestibility of nutrients. These studies were based on an *in vitro* method developed to simulate the pre-caecal digestion in pigs by using commercially available porcine pepsin and pancreatin (Boisen, 2007a,b). The feed used in these studies was the wet feed administered to the dogs in the tolerance trial and a commercial dry feed (kibbles).

The first[35] aimed at examining the effect of the additive on the pre-caecal digestibility of organic matter of dog feed. It was designed as a dose-range finding study that included a control. The results showed improvements on the organic matter digestibility at the recommended level, or higher, compared to the control.

The second[36] and the third[37] studies aimed at examining the effect of the additive when added at the use level under three different conditions; following the *in vitro* method as initially described; adding a pre-digestion period of 3 h; simulated direct addition of the enzymes to the duodenum. A positive control was also considered using a commercially available pancreatin. In the third trial, a dry food (kibbles) was also considered; the use of the dry diet allowed to evaluate the effect in diets with low moisture content. In trials 2 and 3, the samples treated with the additive showed higher organic matter digestibility compared to control, regardless the experimental conditions and feed considered.

The use of *in vitro* methods of different kinds (static/dynamic) to evaluate the digestibility (pre-caecal and total tract) in dogs has been described in the literature. The applicant provided many publications at this respect. Some of these publications review the use of *in vitro* methods and their usefulness to study the digestion in dogs (Harmon, 2007; De Godoy et al., 2016; Deschamps et al., 2022); others provide data on *in vitro* studies only (Van Zelst et al., 2015; Lee et al., 2017; Kim et al., 2021) or do not allow for a full comparison of the results with those obtained *in vivo* (Gajda et al., 2005); a couple describe methods that may not be comparable to the one described by Boisen in 2007 (Smeets-Peeters et al., 1999; Bosch et al., 2016); and some were developed from Boisen's or can be considered comparable but do not provide validation of *in vivo* reference values at pre-caecal level (Dufour-Etienne et al., 1992; Tonglet et al., 2001; Hervera et al., 2007; Hervera et al., 2009; Hooda et al., 2012; Biagi et al., 2016; Penazzi et al., 2021). Some of the latter showed good correlation between the digestibility coefficients in vitro and in vivo (faecal data); the conditions of the studies and diets evaluated may play a major role in the correlations observed.

[35] Technical dossier/Section IV/Annex IV.01.
[36] Technical dossier/Section IV/Annex IV.02.
[37] Technical dossier/Section IV/Annex IV.3.

The results in the three *in vitro* studies show that the additive at the recommended levels can digest wet/dry dog feed under the conditions of the studies. The results support the mode of action of the additive on the digestibility of the organic matter of the dog feed in the foregut.

3.3.2. *In vivo* studies

No studies conducted with the additive under application were submitted.

The applicant submitted several publications reporting studies conducted in dogs in which the use of products like the one under assessment is described; the studies included different enzyme products of animal origin (e.g. pancreas extracts or raw pancreas) and with different formulations (e.g. powder, granulate or coated forms). The studies/data reported were obtained in animals with clinical exocrine pancreatic insufficiency and permitted to see the positive effect of feeding the animals with the enzymes (Wiberg et al., 1998; Rutz et al., 2002; Wiberg and Westermarck, 2002; Snead, 2006; Westermarck and Wiberg, 2012; Mösseler, 2013) or the comparison of different formulations of the enzymes (Mas et al., 2012; Parambeth et al., 2018). The applicant also made reference to the use of similar enzymes in dog diets (e.g. amylase and non-starch polysaccharides; Twomey et al., 2003a,b) or the use of pancreatin in humans and other species (Englyst et al., 1992; de Souza, 2003 and Jiang et al., 2008), as supporting evidence of the efficacy of the enzymes contained in the additive. The applicant considers that the improvements in the digestibility may help clinically healthy dogs in different situations (e.g. impaired (not clinically) exocrine function (e.g. ageing dogs), dogs with high energy requirements (e.g. lactating bitches or sport animals), changes in the diet, etc.). No data have been provided by the applicant at this regard; the applicant also provided ethical/practical reasons for not conducting studies in different animal models (e.g. ageing dogs, lactating bitches).

In a study conducted with healthy dogs, Villaverde et al. (2017) concluded that the supplementation of a maintenance dry diet with pancreas gland extract[38] did not result in an improvement on the digestibility of protein, fat or energy in adult healthy dogs.

The literature results obtained in dogs support the efficacy of feeding pancreatic extracts/tissues to dogs suffering from exocrine pancreatic insufficiency. The study by Villaverde et al. (2017) would cast some doubts on the efficacy of the supplementation of pancreas extract in healthy animals.

3.3.3. Conclusion on efficacy

The results in the three *in vitro* studies show that the additive at the recommended levels can digest wet/dry dog feed under the conditions of the studies. The Panel considers that the results obtained support the mode of action of the additive but that *in vivo* data would be necessary to conclude on the efficacy.

No *in vivo* data were made available with the additive under assessment in dogs not suffering from exocrine pancreatic insufficiency. The FEEDAP Panel cannot conclude on the efficacy of the product as a feed additive in dogs at the recommended conditions of use.

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[39] and Good Manufacturing Practice.

4. Conclusions

The FEEDAP Panel cannot conclude on the safety and the efficacy of Pan-zoot as a feed additive for dogs under the proposed conditions of use.

The FEEDAP Panel cannot conclude on the skin/eye irritancy potential of the additive or on its dermal sensitisation potential. Owing to its proteinaceous nature, the additive is considered a respiratory sensitiser. The additive may induce allergic reactions to the exposed users.

The Panel concludes there is no need for an environmental risk assessment.

[38] (at 71,400 lipase Ph Eur units, 6,208 protease Ph Eur units and 102,222 amylase Ph Eur units per day and animal; enzyme activities that are in a similar range to the ones proposed in the current application except for lipase which represents 60%)

[39] 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.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CFU	colony-forming unit
DM	dry matter
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed