

ADOPTED: 7 January 2020

doi: 10.2903/j.efsa.2020.6205

Safety of 3-phytase FLF1000 and FSF10000 as a feed additive for pigs for fattening and minor growing porcine species

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Abstract

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 3-phytase FLF1000/FSF10000 as a feed additive for pigs for fattening and minor porcine species for growing. This additive contains 3-phytase produced by a genetically modified strain of *Komagataella phaffii* and it is authorised in its liquid FLF1000 and solid form FSF10000 as a feed additive for chickens for fattening, laying hens, chickens reared for laying and for minor poultry species for fattening or reared for laying or for breeding. The FEEDAP Panel has adopted an opinion regarding the use of this product as a feed additive in pigs for fattening and minor porcine species for growing. In that opinion, the FEEDAP Panel concluded that the use of the product as a feed additive would raise no concerns for the consumer safety nor for the environment but that the additive should be regarded as a potential respiratory sensitizer. The Panel also concluded that the additive has a potential to be efficacious in improving the phosphorus utilisation in the target species. However, the Panel could not conclude on the safety for the target species due to the limitations identified in the data provided. The applicant has now complemented the information and made it available to the Panel. The tolerance trial was done in weaned piglets in which the animals received up to 10 times the recommended dose of 1,000 FTU/kg feed. The results showed no adverse effects of the phytase on the performance of the animals or on the haematological and biochemical parameters measured in blood. Therefore, the Panel concluded that the additive (3-phytase FLF1000 and FSF10000) is safe for pigs for fattening and minor growing porcine species at the recommended dose of 1,000 FTU/kg feed.

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Keywords: Zootechnical additive, digestibility enhancers, substances which favourably affect the environment, safety, efficacy, 3-phytase, pigs

Requestor: European Commission

Question number: EFSA-Q-2020-00251

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Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, 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, Dierick NA, Martelli G, Galobart J, Holczknecht O, Manini P, Pettenati E, Pizzo F, Tarrés-Call J and Anguita M, 2020. Scientific Opinion on the safety of 3-phytase FLF1000 and FSF10000 as a feed additive for pigs for fattening and minor growing porcine species. *EFSA Journal* 2020;18(7):6205, 7 pp. <https://doi.org/10.2903/j.efsa.2020.6205>

ISSN: 1831-4732

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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 animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Fertinagro Biotech S.L., is seeking a community authorisation of Preparation of 3-phytase (EC 3.1.3.8) produced by *Pichia pastoris* (ATCC 76273/CBS 7435/CECT 11047) FLF1000 as a feed additive to be used as digestibility enhancers and substances which favourably affect the environment for pigs for fattening and minor porcine species for growing (Table 1).

Table 1: Description of the substances

Category of additive	Digestibility enhancers and substances which favourably affect the environment
Functional group of additive	Digestibility enhancers and substances which favourably affect the environment
Description	Preparation of 3-phytase (EC 3.1.3.8) produced by <i>Pichia pastoris</i> (ATCC 76273/CBS 7435/CECT 11047) FLF1000
Target animal category	Pigs for fattening and minor porcine species for growing
Applicant	Fertinagro Biotech S.L.
Type of request	New opinion

On 3 July 2019, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the safety and efficacy of the product, could not conclude on the safety of Preparation of 3-phytase (EC 3.1.3.8) produced with *Pichia pastoris* (ATCC 76273/CBS 7435/CECT 11047) FLF1000. After the discussion with the Member States on the last Standing Committee, it was suggested to check for the possibility to demonstrate the safety.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment to allow a revision of Authority's opinion. The new data have been received on 7 February 2020.

In view of the above, the Commission asks the Authority to deliver a new opinion on Preparation of 3-phytase (EC 3.1.3.8) produced by *Pichia pastoris* (ATCC 76273/CBS 7435/CECT 11047) FLF1000 as a feed additive for pigs for fattening and minor porcine species based on the additional data submitted by the applicant.

1.2. Additional information

The additive under assessment is presented in two different formulations, liquid (FLF1000) and solid (FSF10000). The additive contains 3-phytase (Enzyme Commission number 3.1.3.8) produced by a genetically modified strain of *Komagataella phaffii* (CECT 13094) and is authorised as a feed additive for chickens for fattening and laying hens,¹ for chickens reared for laying and minor poultry species for fattening or reared for laying or for breeding.^{2,3}

The mandate from European Commission refers to the production strain as *Pichia pastoris* (ATCC 76273/CBS 7435/CECT 11047) FLF1000, however, the production strain of the above-mentioned product should be referred as *K. phaffii*, in line with the current naming convention and data supporting the taxonomic identification submitted in previous applications. The deposit numbers listed

¹ Commission implementing Regulation (EU) 2017/895 of 24 May 2017 concerning the authorisation of a preparation of 3-phytase produced by *Komagataella pastoris* (CECT 13094) as a feed additive for chickens for fattening and laying hens (holder of authorisation Fertinagro 0014 SL). OJ L 138, 25.5.2017, p. 120.

² Commission implementing Regulation (EU) 2019/144 of 28 January 2019 concerning the authorisation of a preparation of 3-phytase produced by *Komagataella pastoris* (CECT 13094) as a feed additive for chickens reared for laying and minor poultry species for fattening or reared for laying or for breeding (holder of authorisation Fertinagro Biotech S.L.). OJ L 27, 31.1.2019, p. 8.

³ Commission implementing Regulation (EU) 2019/781 of 15 May 2019 concerning the authorisation of a preparation of 3-phytase produced by *Komagataella phaffii* (CECT 13094) as a feed additive for chickens for fattening or reared for laying, laying hens and minor poultry species for fattening, for breeding and reared for laying (holder of authorisation Fertinagro Nutrientes S.L.). OJ L 127, 16.5.2019, p. 1.

in the mandate (ATCC 76273/CBS 7435/CECT 11047) are the deposit numbers of the parental strain. The production strain is deposited at the Colección Española de Cultivos Tipo with the deposit number CECT 13094.

The FEEDAP Panel adopted an opinion on the safety and efficacy of the additive 3-phytase FLF1000 (liquid formulation) as a feed additive for chickens for fattening and laying hens (EFSA FEEDAP Panel, 2016) and one on the extension of use in chickens for fattening and minor poultry species (EFSA FEEDAP Panel, 2018). The Panel also evaluated the safety and efficacy of the solid formulation of the additive (3-phytase FSF10000) for the same poultry species (EFSA FEEDAP Panel, 2019a). Later the FEEDAP Panel evaluated the safety and efficacy of the liquid formulation FLF1000 as a feed additive for pigs for fattening (EFSA FEEDAP Panel, 2019b) and also for the solid formulation (EFSA FEEDAP Panel, 2020). This latter opinion included also the request for the use of the solid and liquid formulations (FSF10000 and FLF1000) in turkeys for fattening or reared for breeding.

In the opinions dealing with the safety and efficacy of the product in pigs for fattening and minor growing porcine species, the FEEDAP Panel could not conclude on the safety of the product for the target species (EFSA FEEDAP Panel, 2019b (opinion referred in the mandate for this opinion) and EFSA FEEDAP Panel, 2020). The applicant has provided supplementary data to support the safety of the additive in pigs for fattening and minor growing porcine species.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information⁴ to a previous application on the same additive.⁵

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of the additive is in line with the principles laid down in Regulation (EC) No 429/2008⁶ and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011) and the Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017).

3. Assessment

The additive under assessment contains 3-phytase (Enzyme Commission number 3.1.3.8) and it is available in liquid and solid formulations (FLF1000 and FSF10000). The 3-phytase is produced by a genetically modified strain of *K. phaffii* (CECT 13094). In previous opinions, the Panel described the additive and its manufacturing process including the production strain and its genetic modification (EFSA FEEDAP Panel, 2016, 2019a). The liquid formulation ensures a minimum phytase activity of 1,000 FTU/mL and the solid one of 10,000 FTU/g additive.

The additive in either form is intended to be used in feed for pigs for fattening and minor porcine species for growing at 1,000 FTU/kg feed.

The FEEDAP Panel adopted two opinions regarding the use of this additive in pigs for fattening and minor porcine species (EFSA FEEDAP Panel, 2019b, 2020). In those assessments, the applicant provided a combined tolerance and efficacy trial in weaned piglets to support the safety for pigs for fattening and other minor porcine species for growing.⁷ However and owing to the lack of precise data on the total feed intake of the animals, the FEEDAP Panel could not consider further the study for the assessment and consequently no conclusion could be drawn regarding the safety of the additive for pigs for fattening nor for other minor growing porcine species.

The applicant has provided supplementary data regarding the feed intake of the animals under study and the study is presented below.

⁴ FEED dossier reference: FAD-2020-0014.

⁵ FEED dossier reference: FAD-2017-0023.

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 FAD-2017-0023 Technical dossier/Section III/Annex III.1.

3.1. Safety for pigs for fattening

A total of 150 weaned male piglets (Pietrain × ACMC-Meidam, 46 days of age, body weight 12.3 kg) were housed in pens in groups of five piglets each and allocated to six dietary treatments (representing five replicate pens per treatment).⁷ The experiment was conducted in five runs, including 30 piglets per run (one pen with five piglets per treatment). Before the start of the tolerance trial, the piglets had a 11-day (from 35 to 45 days of age). A basal diet based on maize, wheat, barley and soya bean meal (total P content 0.47% and Ca content 0.78%) was either not supplemented (control) or supplemented with 3-phytase FLF1000 to provide 250, 500, 1,000 (1 × recommended dose) or 10,000 (10 × recommended dose) FTU/kg feed. Enzyme activities were confirmed by analysis. A positive control with a higher P content was also considered (total P content 0.58% and Ca content 0.77%). Feeds in mash form and water were offered ad libitum for 49 days. Health status and mortality were monitored daily and most probable cause of death determined by necropsy. From day 7 to day 18 of the experiment, a digestibility trial was performed with 20 piglets per treatment. Four piglets per pen were placed in two metabolic cages (2 pigs per cage) and after the digestibility trial the animals were placed back to the pen.

The feed intake was measured throughout the study period. In the previous submission, the intake was reported for periods 1–7 days and 18–49 days under study as mean values per pen (of 5 animals), while for the period 7–18 days under study as mean values per cage (2 animals per cage). The total feed intake per animal was calculated for the overall period in the current submission.⁸ Body weight was recorded at days 1, 7, 14, 18, 28, 35 and 49 of the experiment. Feed to gain ratio was calculated for the different periods. On day 42 of the experiment, blood samples were obtained from ten pigs per treatment (2 pigs per treatment per run) for routine blood haematology and biochemistry.⁹ An analysis of variance (ANOVA) was performed with the data considering the pen or the pig (blood analysis) as the experimental unit, group means were compared by Dunnett's test and polynomial orthogonal contrasts were used to test for linear effects of the phytase, excluding the data for the positive control. The significance level was set at $p < 0.05$.

Two animals died during the study (mean mortality value of 1.3%); one piglet from the control diet and another one from the 500 FTU/kg feed group. No differences among treatments were observed in feed intake (1,079, 1,085, 1,090, 1,122, 1,128 and 1,083 g/day for control, 250, 500, 1,000, 10,000 and the positive control, respectively). Final body weight was 40.9, 41.8, 41.8, 45.3 and 46.1 and 43.7 kg for control, 250, 500, 1,000, 10,000 and the positive control, respectively, and the groups 1,000 and 10,000 FTU/kg feed showed significantly higher body weights compared to the control diet (significant linear effect $p < 0.05$). These results indicate no adverse effects of the additive on the performance of the animals.

The haematological parameters measured showed significant effects of the diet on the haematocrit that were random modifications and not dose related. The biochemical parameters analysed in blood showed significant differences between the treatments for phosphorus and calcium concentration, alkaline phosphatase and albumin. For phosphorus, the control showed the lowest value (7.84 mg/dL) and was significantly different compared to 10,000 FTU/kg (9.95), while for calcium concentration the control group showed the highest value (13.02 mg/dL) and was significantly different to 10,000 FTU/kg (11.42). The control showed the highest value of alkaline phosphatase (259 U/L) and was significantly different to 250 FTU/kg and 10,000 FTU/kg (209 and 176). In the case of the albumin, the values were significantly lower in the control compared to 500 FTU/kg and 1,000 FTU/kg (control 3.49 g/dL vs. 3.81 and 3.83 g/dL, respectively) but no differences were observed between the control and the overdosed group (10,000 FTU/kg). Polynomial contrasts showed non-significant linear effect for any of these parameters, except for a significant effect on albumin (and total protein) and a trend in alkaline phosphatase. These modifications in the biochemical parameters show effects that can be expected from the use of the phytase (phosphorus, alkaline phosphatase) and/or did not show a dose response and consequently raise no concerns.

The supplementation of the phytase up to 10-fold the recommended dose of 1,000 FTU/kg feed did not show adverse effects on the performance of the piglets or on the blood parameters measured. Therefore, the FEEDAP Panel considers that the additive is safe for pigs for fattening at the recommended dose of 1,000 FTU/kg feed with a margin of safety of at least 10-fold. This conclusion

⁸ FAD-2020-0014/Suppl. Information.

⁹ Including: red blood cells, haemoglobin, hematocrit, platelets, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cells (including differential counts), glucose, calcium, phosphorus, alkaline phosphatase, alanine transaminase, aspartate transaminase, total bilirubin, amylase, gamma-glutamyl transpeptidase, uric acid, creatinine, albumin and total protein.

can be extrapolated to minor growing porcine species. The Panel considers that this conclusion applies to the two forms of the additive.

4. Conclusions

The additive in either form is safe for pigs for fattening and minor growing porcine species at the recommended dose of 1,000 FTU/kg feed.

5. Documentation as provided to EFSA/Chronology

Date	Event
12/02/2020	Dossier received by EFSA. Supplementary dossier on 3-phytase FLF1000 as a feed additive for pigs for fattening and minor porcine species for growing. Fertinagro Biotech SA
20/02/2020	Reception mandate from the European Commission
23/03/2020	Application validated by EFSA – Start of the scientific assessment
01/07/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ANOVA analysis of variance

FEEDAP EFSA Panel on Additives and Products or Substances used in Animal Feed