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

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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 and efficacy of 3-phytase FLF1000 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 the European Union as a feed additive for feed 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 applicant requested the extension of use of the additive to pigs for fattening and minor porcine species for growing. The FEEDAP Panel concluded that the use of the product as a feed additive for pigs raises no concerns for the consumer safety nor for the environment. The additive should be regarded as a potential respiratory sensitiser. The applicant provided a combined tolerance and efficacy trial in weaned piglets to support the safety for the target species. However, owing to the lack of precise data on the total feed intake of the animals, the FEEDAP Panel did 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. Three studies were considered for the efficacy and from the data obtained the FEEDAP Panel concluded that the additive has the potential to be efficacious in improving the phosphorus utilisation in pigs for fattening at a minimum level of 500 FTU/kg feed.

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

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

The European Commission received a request from Fertinagro Nutrientes S.L.[2] for authorisation of the product 3-phytase FLF1000 (3-phytase), when used as a feed additive for pigs for fattening and minor porcine species for growing (category: zootechnical additive; functional groups: digestibility enhancers and substances which favourably affect the environment).

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 7 November 2017.

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 product 3-phytase FLF1000 (3-phytase), when used under the proposed conditions of use.

1.2. Additional information

The additive 3-phytase FLF1000 is a liquid product that contains 3-phytase (Enzyme Commission number 3.2.1.8) produced by a genetically modified strain of *Komagataella phaffii* (CECT 13094)[3] and is authorised as a feed additive for chickens for fattening and laying hens,[4] for chickens reared for laying and minor poultry species for fattening or reared for laying or for breeding.[5] The product is also authorised in the solid form for the same species/categories.[6]

The FEEDAP Panel adopted an opinion on the safety and efficacy of the additive 3-phytase FLF1000 as a feed additive for chickens for fattening and laying hens (EFSA FEEDAP Panel, 2016), another one on the extension of use to chickens reared for laying and minor poultry species (EFSA FEEDAP Panel, 2018) and a last one on the safety and efficacy of the solid formulation of the additive (3-phytase FSF10000) for the species assessed previously (EFSA FEEDAP Panel, 2019). The applicant is now requesting for an extension of the use of the additive to pigs for fattening and minor porcine species for growing.

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[7] in support of the authorisation request for the use of 3-phytase FLF1000 as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

[2] Fertinagro Nutrientes S.L., Pol. Ind. La Paz parcela 185, 44195 Teruel, Spain. The name of the applicant was modified to Fertinagro Biotech, S.L.

[3] Formerly classified as *Komagataella pastoris*. The accession number for the strain presented in the mandate referred to the recipient strain and not to the production strain which is (CECT 13094).

[4] 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.

[5] 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.

[6] 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.

[7] FEED dossier reference: FAD-2017-0023.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the active substance in animal feed are valid and applicable for the current application.[8]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of 3-phytase FLF1000 is in line with the principles laid down in Regulation (EC) No 429/2008[9] and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012) and Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011).

3. Assessment

This assessment deals with a request from the applicant to extend the use of the additive 3-phytase FLF1000 to pigs for fattening and minor porcine species for growing as a zootechnical additive (functional groups: digestibility enhancers and substances which favourably affect the environment).

3.1. Characterisation

The additive 3-phytase FLF1000 in its liquid formulation contains 3-phytase (Enzyme Commission number 3.1.3.8) produced by a genetically modified strain of *K. phaffii* (CECT 13094) with a minimum phytase activity of 1,000 FTU/mL. In a previous opinion, the Panel described the additive and its manufacturing process including the production strain and its genetic modification (EFSA FEEDAP Panel, 2016).

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

3.2. Safety

Safety aspects regarding the use of this additive in feed including the safety of the production strain, the safety for the consumer, for the users and for the environment have been previously evaluated (EFSA FEEDAP Panel, 2016). The FEEDAP Panel concluded that there are no concerns regarding the genetic modification of the production strain, that there are no concerns for the consumer safety and no risks for the environment are expected from the use of the product as a feed additive. Regarding the safety for the user, it was concluded that the additive is not irritant to eyes and skin and is not a dermal sensitiser; however, it should be considered a potential respiratory sensitiser.

The Panel is not aware of any new information that would lead it to reconsider the conclusions drawn previously. Moreover, the FEEDAP Panel considers that the new use requested by the applicant would not modify the above conclusions.

The applicant provided a combined tolerance and efficacy trial in weaned piglets to support the safety for the target species.[10] The trial included a balance trial. The piglets were under study for 48 days. From days 1 to 7 and from days 18 to 48, the piglets were kept in pens with 5 animals. From days 7 to 18, the balance study took place and four animals from each pen were placed in metabolic cages while one pig remained in the original pen and was not included in the balance. The feed intake was measured throughout the study, but the data regarding the overall feed intake was not made available by the applicant. Owing to the lack of precise data on the total feed intake of the animals, the FEEDAP Panel cannot consider further the study for the assessment and consequently no conclusion can be drawn regarding the safety of the additive for pigs for fattening nor for other minor growing porcine species.

3.3. Efficacy for pigs for fattening

A total of five efficacy trials were submitted by the applicant. Two of the trials submitted were not considered: One was the tolerance trial which was conducted with weaned piglets[11] and, therefore,

[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finirep-fad-2015-0026-preparation-3phytase.pdf

[9] 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.

[10] Technical dossier/Section III/Annex III.1.

[11] Technical dossier/Section IV/Annex IV.1.

cannot be considered in the assessment of the efficacy for pigs for fattening; the second one was a long-term trial which was not considered further in the assessment due to the extensive therapeutic treatment the animals received during the study (at least three periods of five-day medication).[12] The other three short-term studies were considered for the assessment and are described below.

The first was a balance trial in which a total of 75 castrated male pigs (PIC line 65 × PIC Camborough 22, body weight 34.3 kg) were allocated to five dietary treatments.[13] Pigs were distributed to pens in groups of three pigs each during the first 9 days of the experiment and then two pigs per pen (10 pigs per treatment) were selected and housed individually in metabolic cages for a period of 9 days to conduct the balance trial. A basal diet based on wheat, barley and soya bean meal (total phosphorus content 0.35% and calcium content 0.78%) was either not supplemented (control) or supplemented with 3-phytase to provide 250, 500 or 1,000 FTU/kg feed. Enzyme activities were confirmed by analysis. A positive control was also considered (total phosphorus content 0.48% and calcium content 0.95%). Feed was offered on *ad libitum* basis in mash form. Health status and mortality were monitored daily. Feed intake was measured and feed to gain ratio was calculated. The balance study consisted of an adaption period of 5 days and a collection period of 4 days for total collection of faeces and urine, separated.

The second was a balance trial in which a total of 60 male pigs (Piétrain × (Large White × Landrace), initial body weight 56.5 kg) were distributed to pens in groups of six pigs each and allocated to five dietary treatments.[14] Pigs were distributed to pens in groups of two pigs each during the first 7 days of the study, then after two pigs per pen were selected and individually housed in metabolic cages (12 pigs per treatment). A basal diet based on wheat, barley and soya bean meal (total phosphorus content 0.49–0.57% and calcium content 0.48–0.60%) was either not supplemented (control) or supplemented with 3-phytase to provide 250, 500 or 1,000 FTU/kg feed. Enzyme activities were confirmed by analysis. A positive control was also considered (total P content 0.65% and Ca content 0.66%). All experimental diets contained titanium dioxide as an external marker. Feeds were offered on *ad libitum* basis in mash form. Health status and mortality were monitored daily. The balance study consisted of an adaption period of 3 days and a collection period of 3 days for total collection of faeces and urine, separated.

The third trial was a digestibility trial in which measurements of the bone ash content were done.[15] A total of 18 male pigs (Duroc × (Landrace × Large White)), initial body weight 35 kg) were allocated to two dietary groups. The pigs were selected according to body weight and distributed to pens in which stayed for 18 days. Then, they were housed individually in metabolic cages for 10 days (9 pigs per treatment). A basal diet based on maize and soya bean meal (total phosphorus content 0.36% and calcium content 0.49%) was either not supplemented (control) or supplemented with 3-phytase to provide 500 FTU/kg feed. Enzyme activities were confirmed by analysis. All experimental diets contained titanium dioxide as external marker. Feed was offered on *ad libitum* basis during the adaptation period and restricted to 2.5 times the energy requirements for maintenance according to the body weight measured during the digestibility trial. The digestibility trial consisted of a 6-day adaptation period and 4 days of total collection of faeces.

In all trials, health status and mortality were monitored daily. Body weight and feed intake were measured throughout the study period. Diets and faeces were analysed to study the digestibility of phosphorus, in trials 1 and 2 urine was also analysed to study the retention of phosphorus. In the third trial all animals were killed at the end of the study and the III and IV metacarpal bones from the left forelimb were collected from all animals at the end of the study and bone ash and phosphorus content were measured. An analysis of variance was done with the data from each study, in trial 1 group means were compared against the control using Dunnett's test and in trial 2 group means were compared using Tukey's test.

The results of the phosphorus utilisation and bone ash content are presented in Table 1. In trials 1 and 2, pigs receiving the additive from 500 FTU/kg or 250 FTU/kg, respectively, showed a higher retention of phosphorus. In trial 3, piglets receiving 500 FTU/kg feed showed a higher digestibility of phosphorus and a higher bone content of ash and phosphorus compared to control.

[12] Technical dossier/Section IV/Annex IV 4.
[13] Technical dossier/Section IV/Annex IV 2.
[14] Technical dossier/Section IV/Annex IV 3.
[15] Technical dossier/Supplementary information April 2019/Annex 2.

Therefore, the FEEDAP Panel concludes that the additive has the potential to be efficacious in improving the phosphorus utilisation in pigs for fattening at a minimum level of 500 FTU/kg feed.

Table 1: Effect of 3-phytase FLF1000 on the apparent phosphorus digestibility/retention and bone mineralisation

Trial	Group FTU/kg feed	Phosphorus (%)			Bone content (%)	
		Digestibility Total collection	Digestibility Marker method	Retention	Ash	Phosphorus
1	Control	41.8		41.5		
	250	45.6		45.2		
	500	48.1*		47.8*		
	1,000	53.1*		52.8*		
	Positive control	54.2*		53.9*		
2	Control	53.7^{b}	46.4^{b}	52.4^{b}		
	250	63.2^{a}	51.3^{ab}	61.8^{a}		
	500	61.3^{a}	56.7^{a}	60.2^{a}		
	1,000	62.2^{a}	52.9^{ab}	60.0^{a}		
	Positive control	59.9^{a}	56.7^{a}	53.7^{b}		
3	Control	45.4^{b}	26.5^{b}	–	37.0^{b}	6.27^{b}
	500	55.1^{a}	38.2^{a}	–	39.0^{a}	6.61^{a}

*: In trial 1, values within one column are statistically significant compared to control ($p < 0.05$).
^{a,b}Values within one trial and within one column with different superscript are statistically different.

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[16] and Good Manufacturing Practice.

4. Conclusions

The FEEDAP Panel cannot conclude on the safety of the additive for pigs for fattening nor for minor growing porcine species.

The FEEDAP Panel concludes that the use of the product as a feed additive raises no concerns for the consumer safety nor for the environment. The additive should be regarded as a potential respiratory sensitiser.

The FEEDAP Panel concludes that the additive has the potential to be efficacious in pigs for fattening at 500 FTU/kg feed.

5. Documentation as provided to EFSA/Chronology

Date	Event
20/04/2017	Dossier received by EFSA. 3-phytase for pigs for fattening. Submitted by Fertinagro Biotech, S.L.
29/05/2019	Reception mandate from the European Commission
07/11/2017	Application validated by EFSA – Start of the scientific assessment
11/12/2017	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for the consumer*
07/02/2018	Comments received from Member States
26/04/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
03/07/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

[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.

References

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. https://doi.org/10.2903/j.efsa.2011.2175

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. https://doi.org/10.2903/j.efsa.2012.2536

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. Scientific opinion on the safety and efficacy of 3-phytase FLF1000 as feed additive for chickens for fattening and laying hens. EFSA Journal 2016;14(11):4622, 15 pp. https://doi.org/10.2903/j.efsa.2016.4622

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2018. Scientific Opinion on the safety and efficacy of 3-phytase FLF1000 as a feed additive for chickens reared for laying and minor poultry species. EFSA Journal 2018;16(3):5203, 6 pp. https://doi.org/10.2903/j.efsa.2018.5203

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2019. Scientific Opinion on the safety and efficacy of 3-phytase FSF10000 as a feed additive for chickens for fattening or reared for laying, laying hens and minor poultry species. EFSA Journal 2019;17(1):5543, 10 pp. https://doi.org/10.2903/j.efsa.2019.5543

Abbreviations

EURL	European Union Reference Laboratory
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed