

ADOPTED: 23 March 2023

doi: 10.2903/j.efsa.2023.7973

## **Efficacy of a feed additive consisting of 6-phytase produced by *Komagataella phaffii* CGMCC 12056 (APSA PHYTAFEED® 20,000 GR/L) for pigs for fattening (Andrés Pintaluba S.A)**

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Daniel Pagés Plaza and Jordi Ortuño Casanova

### **Abstract**

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the efficacy of 6-phytase produced by the genetically modified strain *Komagataella phaffii* CGMCC 12056 (APSA PHYTAFEED® 20,000 GR/L) as a zootechnical feed additive (functional group: digestibility enhancers) for pigs for fattening at a minimum recommended level of 1,000 U /kg feed. In a previous assessment, three trials were submitted; one of them showed significant improvements on zootechnical parameters supporting the efficacy of the additive. The other two trials evaluating apparent faecal phosphorus digestibility and bone mineralisation, showed no improvements on phosphorus retention in bone and, therefore, the FEEDAP Panel could not conclude on the efficacy of the additive. The applicant submitted two new efficacy studies in pigs for fattening evaluating the animal's zootechnical performance, apparent faecal phosphorus digestibility and bone mineralisation. One of the trials was not considered due to high rates of mortality, culling of animals and medical treatments applied. In the other trial, significant improvements on final body weight and apparent total track phosphorus digestibility were found in the animals that received the phytase at 1,000 U/kg complete feed. Owing to the lack of sufficient data, the FEEDAP Panel could not conclude on the efficacy of the additive in pigs for fattening.

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**Keywords:** safety, efficacy, zootechnical additives, digestibility enhancers, phytase, pigs for fattening, APSA PHYTAFEED® 20,000 GR/L

**Requestor:** European Commission

**Question number(s):** EFSA-Q-2022-00507

**Correspondence:** [feedap@efsa.europa.eu](mailto:feedap@efsa.europa.eu)

**Panel members:** Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

**Declarations of interest:** If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact [interestmanagement@efsa.europa.eu](mailto:interestmanagement@efsa.europa.eu).

**Acknowledgements:** The Panel wishes to thank the following for the support provided to this scientific output (in alphabetical order of the last name): Montserrat Anguita Freixa.

**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, Fašmon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Pagés Plaza D and Ortuño Casanova J, 2023. Scientific Opinion on the efficacy of a feed additive consisting of 6-phytase produced by *Komagataella phaffii* CGMCC 12056 (APSA PHYTAFEED® 20,000 GR/L) for pigs for fattening (Andrés Pinaluba S.A). EFSA Journal 2023;21(4):7973, 7 pp. <https://doi.org/10.2903/j.efsa.2023.7973>

**ISSN:** 1831-4732

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



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## 1. Introduction

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

Regulation (EC) No 1831/2003<sup>1</sup> establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant Andrés Pinaluba S.A.<sup>2</sup> is seeking a community authorisation of 6-phytase as a feed additive to be used as a zootechnical additive for pigs for fattening (Table 1).

**Table 1:** Description of the additive

Category of additive	Zootechnical additive
Functional group of the additive	Digestibility enhancers
Description	6-phytase
Target animal category	Pigs for fattening
Applicant	Andrés Pinaluba S.A.
Type of request	New opinion

On 20 January 2020,<sup>3</sup> the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of the European Food Safety Authority (EFSA), in its opinion on the safety and efficacy of the product, could not conclude on the efficacy of 6-phytase in pigs for fattening.

The Commission gave the possibility to the applicant to submit supplementary information and data in order to complete the assessment and to allow a revision of the EFSA's opinion. The new data have been received on 1 August 2022 and the applicant has been requested to transmit them to EFSA as well.

In view of the above, the Commission asks EFSA to deliver a new opinion on 6-phytase as a feed additive for pigs for fattening, based on the supplementary data submitted by the applicant, in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002.

### 1.2. Additional information

The additive, with the trade name APSA PHYTAFEED® 20,000 GR/L P, is a preparation containing 6-phytase currently authorised in growing poultry species, laying hens and other laying birds, piglets (suckling and weaned) and minor porcine species.<sup>4,5,6</sup>

The FEEDAP Panel issued five opinions on the safety and the efficacy of APSA PHYTAFEED® 20,000 GR/L (6-phytase) in different target species (EFSA FEEDAP Panel, 2019a,b,c, 2020a,b). In the last opinion, the Panel could not conclude on the efficacy of the additive in pigs for fattening.

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information<sup>7</sup> to a previous application on the same product.<sup>8</sup>

<sup>1</sup> 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.

<sup>2</sup> Andres Pinaluba S.A. Pol. Ind. Agro Reus, c/ Prudenci Bertrana, 5, Reus 43206, Spain.

<sup>3</sup> It is noted that the adoption date was 12 of December 2019.

<sup>4</sup> Commission Implementing Regulation (EU) 2020/150 of 4 February 2020 concerning the authorisation of the preparation of 6-phytase produced by Komagataella phaffii CGMCC 12056 as a feed additive for chickens for fattening, chickens reared for laying and for breeding and minor poultry species for fattening or reared for laying or for breeding purposes (holder of authorisation Andrés Pinaluba S.A.) OJ L 33, 5.2.2020, p. 9–11.

<sup>5</sup> Commission Implementing Regulation (EU) 2020/1376 of 1 October 2020 concerning the authorisation of a preparation of 6-phytase, produced by Komagataella phaffii (CGMCC 12056) as feed additive for turkeys for fattening, turkeys reared for breeding, piglets (suckling and weaned) and minor porcine species (holder of the authorisation Andrés Pinaluba S.A.) C/2020/6612.

<sup>6</sup> Commission Implementing Regulation (EU) 2020/1799 of 30 November 2020 concerning the authorisation of a preparation of 6-phytase produced by Komagataella phaffii CGMCC 12056 as a feed additive for laying hens and other laying birds (holder of authorisation: Andrés Pinaluba S.A.) OJ L 402, 1.12.2020, p. 43–45.

<sup>7</sup> Dossier reference: EFSA-Q-2022-0050.

<sup>8</sup> Dossier reference: FAD-2019-0021.

In accordance with Article 38 of the Regulation (EC) No 178/2002<sup>9</sup> and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,<sup>10</sup> a non-confidential version of the supplementary information has been published on Open.EFSA.<sup>11</sup>

## 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of active substance (trade name of the product) is in line with the principles laid down in Regulation (EC) No 429/2008<sup>12</sup> and the relevant guidance documents: Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

## 3. Assessment

APSA PHYTAFEED® 20,000 GR/L is a preparation containing 6-phytase produced by a genetically modified strain of the yeast *Komagataella phaffii* (CGMCC 12056) that has been deposited in the China General Microbiological Culture Collection Centre (CGMCC) with the deposit number 12056.

The additive is available in two formulations, a solid one, (APSA PHYTAFEED® 20,000 GR), and a liquid one, (APSA PHYTAFEED® 20,000 L). Both formulations of the additive ensure a guaranteed minimum phytase activity of 20,000 U/g or mL of product.

The additive is intended to be used as a zootechnical additive (functional group: digestibility enhancers) in feed for pigs for fattening at a minimum recommended level of 1,000 U<sup>13</sup>/kg feed.

In previous opinions, the FEEDAP Panel concluded that the additive is safe for the target species, consumer, user and the environment (EFSA FEEDAP Panel, 2019a, 2020a). However, the data provided were not sufficient to conclude on the efficacy of the additive in pigs for fattening. In the last opinion, one long-term trial evaluating the zootechnical performance parameters in pigs and two short-term digestibility studies that included bone measurements were submitted to support the efficacy of the additive (EFSA FEEDAP Panel, 2020a). The results of the long-term trial showed significant improvements in the zootechnical performance of pigs for fattening receiving the phytase at 1,000 U/kg complete feed. The two digestibility trials significantly improved the apparent faecal digestibility of phosphorus in pigs fed the additive. However, no significant improvements on the bone mineralisation were observed and, therefore, the studies did not support the efficacy of the additive. Owing to the limited evidence, the FEEDAP Panel could not conclude on the efficacy of the additive in pigs for fattening.

The applicant provided two additional trials to complement the previous assessment.

### 3.1. Efficacy

The applicant submitted two long-term trials studying the effect of the additive on the zootechnical performance of the animals, apparent faecal phosphorus digestibility and bone mineralisation. In the first study,<sup>14</sup> most of the animals were treated with antibiotics (> 94%) and the mortality and culling rates were high (9.1% on average). Therefore, this study was not further considered for the assessment.

In the second study,<sup>15</sup> a total of 352 pigs for fattening<sup>16</sup> (14-week-old; average initial body weight 30.65 kg) were distributed in 32 pens (16 males, 16 females) and were randomly allocated to 4 dietary treatments (8 replicates per treatment; 11 animals per replicate).

<sup>9</sup> Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

<sup>10</sup> Decision available online: <https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements>

<sup>11</sup> Available online: <https://open.efsa.europa.eu/questions/EFSA-Q-2022-00507>

<sup>12</sup> 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.

<sup>13</sup> One Unit (U) is defined as the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute at pH 5.5 and 37°C.

<sup>14</sup> 'Appendix\_3\_efficiencytrial\_TTPIN22001'.

<sup>15</sup> 'Appendix\_2\_efficiencytrial\_CLIO471\_1C'.

<sup>16</sup> (Pietrain × (Landrace × Large White)).

Two basal diets (grower, from days 1 to 49; finisher, from days 50 to 75) based on maize, wheat and soya bean were either not supplemented (control) or supplemented with APSA PHYTAFEED® 20,000 GR to provide 250 U/kg complete feed or 1,000 U/kg complete feed (confirmed by analysis). A fourth group of pigs was included, receiving a diet with standard content of phosphorus and calcium (positive control). The diets were offered in mash form on ad libitum basis for 75 days. The finisher diets included an external marker for the digestibility studies. Mortality and health status were checked every day and the most likely reason of death/culling provided. The animals were individually weighed at the start of the trial (day 1). Thereafter, the average body weight and feed intake were recorded on pen basis at days 21, 42, 70 and 74 and average daily feed intake (ADFI), average daily weight gain (ADG) and feed to gain ratio (F:G) were calculated and corrected for mortality for the overall period. During the days 71–74, faecal rectal samples were collected and pooled per pen. Feed and faecal samples were analysed for the content of the external marker, calcium (Ca) and phosphorus (P) to calculate their apparent total tract digestibility. On day 75, one pig per pen (the one with the body weight closest to the mean value of the pen) was killed and samples of the right III metacarpal bone were collected, weighed, and analysed for ash, Ca and P. Data were analysed with a general linear model with the treatment and sex as fixed effects. Mean groups were compared using Tukey’s test. Significance level was set at  $p < 0.05$ .

The overall mortality was 2.27% on average. The results on the performance parameters are reported in Table 2 and apparent total tract digestibility (ATTD) and bone mineralisation in Table 3.

**Table 2:** Effect of APSA PHYTAFEED® 20,000 GR/L on the zootechnical parameters (days 1–74)

Phytase (units/kg feed)	Daily feed intake	Final body weight	Average daily weight gain	Feed to gain ratio
	(kg)	(kg)	(kg)	
0	1.85	95.0 <sup>b</sup>	0.87	2.12
250	1.86	96.7 <sup>b</sup>	0.88	2.12
1,000	1.88	101.5 <sup>a</sup>	0.90	2.08
Positive control	1.86	98.9 <sup>a,b</sup>	0.89	2.08

a,b: Mean values within a column with a different superscript are significantly different  $p < 0.05$ .

**Table 3:** Effect of APSA PHYTAFEED® 20,000 GR/L on the apparent phosphorus digestibility and metacarpal bone content of ash and phosphorus (days 71–74)

Phytase (units/kg feed)	Ca and P content in feed	ATTD	Bone content	
			Ash	P
	(%Ca/%P)	(%)	(%)	(% of ash)
0	0.58/0.42	32.1 <sup>b</sup>	23.5	18.0
250	0.58/0.42	51.2 <sup>a</sup>	24.4	18.1
1,000	0.58/0.42	47.8 <sup>a</sup>	25.3	18.2
Positive control	0.75/0.54	41.4 <sup>a,b</sup>	25.4	17.9

ATTD: apparent total tract digestibility.

a,b: Mean values within a column with a different superscript are significantly different  $p < 0.05$ .

The results showed a significant improvement of the zootechnical performance (higher final body weight) of the pigs in the group supplemented with 1,000 U/kg complete feed compared to the control. These results were supported by higher apparent faecal digestibility of phosphorus in pigs fed the diets supplemented with the additive. No significant improvements on the bone mineralisation were observed.

After considering all the data provided by the applicant, two-long term trials in pigs for fattening showed positive effects in the zootechnical performance at 1,000 U/kg complete feed.

#### 4. Conclusions

Owing to the lack of sufficient data, the FEEDAP Panel is not in the position to conclude on the efficacy of the additive APSA PHYTAFEED® 20,000 GR/L for pigs for fattening.

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## Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ATTD	apparent total tract digestibility
CGMCC	China General Microbiological Culture Collection Centre
EURL	European Union Reference Laboratory
F:G	feed to gain Ratio