SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of 3-phytase produced by Komagataella phaffii (CECT 13171) (FSF10000/FLF1000) for poultry species, pigs for fattening and minor porcine species (FERTINAGRO BIOTECH S.L.)

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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 3-phytase produced by Komagataella phaffii (CECT 13171) (FSF10000/FLF1000) as a zootechnical additive for poultry species, pigs for fattening and minor porcine species. The production strain (CECT 13171) is genetically modified and was developed from a strain that had been previously assessed by the FEEDAP Panel. The genetic modifications present in K. phaffii CECT 13171 do not raise safety concerns and no recombinant DNA was detected in the final formulations of the additive (FSF10000/FLF1000). However, the Panel could not conclude on the identity of the production strain and uncertainty remained on the possible presence of viable cells of the production strain in the final formulations. Owing to these uncertainties, the FEEDAP Panel could not conclude on the safety of the additive regarding the production strain. The additive in either form is not irritant to eyes and skin, the liquid formulation is not a dermal sensitiser but the solid formulation is, and the two formulations should be considered potential respiratory sensitisers. The FEEDAP Panel concluded that the 3-phytase present in the additive is safe for the target species at a level of 1,000 FTU/kg feed and that would not raise safety concerns for the environment. However, considering the uncertainties on the identification of the production strain and the possible presence of viable cells in the final formulations, the Panel could not conclude on the safety of the additive for the target species, consumer, users, and the environment. The FEEDAP Panel concluded that the additive is efficacious at 500 FTU/kg feed in poultry species for fattening or reared for laying/breeding, pigs for fattening and minor porcine species and at 1,000 FTU/kg feed in laying hens.

Keywords: zootechnical additives, digestibility enhancers, substances which favourably affect the environment, 3-phytase, safety, efficacy, poultry, 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 feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from (Fertinagro Biotech S.L.)\(^2\) for the authorisation of the additive consisting of 3-phytase produced by *Komagataella phaffii* CECT 13171 (FSF10000/FLF1000), when used as a feed additive for chickens for fattening/reared for laying, laying hens, turkeys for fattening/reared for breeding, minor poultry species, pigs for fattening and other suidae (minor porcine species for fattening) (category: zootechnical additives; functional group: 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). The particulars and documents in support of the application were considered valid by EFSA as of 4 June 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 3-phytase produced by *K. phaffii* CECT 13171 (FSF10000/FLF1000), when used under the proposed conditions of use (see Section 3.1.3).

1.2. **Additional information**

The additive contains 3-phytase (Enzyme Commission number 3.2.1.8) and is available in solid and liquid forms (named as FSF10000 and FLF1000). The 3-phytase is produced by a genetically modified strain of *K. phaffii* which is deposited at the Spanish Type Culture Collection with the deposition number CECT 13171. The production strain is derived from another *K. phaffii* genetically modified strain (CECT 13094) that has been previously assessed by the FEEDAP Panel (see below) and produces the same 3-phytase.

The FEEDAP Panel adopted several opinions on the safety and efficacy of the 3-phytase produced by *K. phaffii* (CECT 13094), from the same applicant as the current application. The first was 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); the second was on the extension of use in chickens for fattening and minor poultry species (EFSA FEEDAP Panel, 2018a). The Panel also evaluated the safety and efficacy of the solid formulation of the additive (FSF10000) for the same poultry species (EFSA FEEDAP Panel, 2019a). Later the FEEDAP Panel evaluated the safety and efficacy of the liquid and solid formulations as a feed additive for pigs for fattening and in turkeys for fattening or reared for breeding (EFSA FEEDAP Panel, 2019b, 2020a,b).

The additive 3-phytase produced by a genetically modified strain of *K. phaffii* (CECT 13094) is authorised as a feed additive for chickens for fattening and laying hens, for chickens reared for laying

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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 FERTINAGRO BIOTECH S.L., Poligono Industrial La Paz Parcela 185, 44195, Teruel, Spain.
and minor poultry species for fattening or reared for laying or for breeding, turkeys for fattening or reared for breeding, pigs for fattening and minor porcine species for fattening.3,4,5,6

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier7 in support of the authorisation request for the use of 3-phytase produced by a genetically modified strain, K. phaffii CECT 13171, 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. 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 The FEEDAP Panel notes that the applicant changed the conditions of use established at the time of application for laying hens. The change was done during the validation process of the dossier and consisted in lowering of the level from 1,000 to 375 FTU/kg feed. The previous evaluation of the EURL was done considering a minimum level of phytase in the diet of 500 FTU/kg feed. However, the method proposed by the applicant for the evaluation in feed is EN ISO 30024 method which should ensure fitness for the newly requested level.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy 3-phytase produced by the genetically modified strain K. phaffii CECT 13171 (FSF10000/FLF1000) is in line with the principles laid down in Regulation (EC) No 429/20089 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, 2018b), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018c) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019c).

3. Assessment

The additive is available in solid and liquid forms, referred herein and after as FSF10000 and FLF1000, respectively, contains 3-phytase (Enzyme Commission number 3.2.1.8) and is intended to be used as a zootechnical feed additive in feed for poultry species for fattening or reared for laying/breeding, laying hens, pigs for fattening and minor porcine species for growing.

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3 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 6014 SL). OJ L 138, 25.5.2017, p. 120.
4 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.
5 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 Nutrients S.L.). OJ L 127, 16.5.2019, p. 1.
6 Commission Implementing Regulation (EU) 2021/330 of 24 February 2021 concerning the authorisation of a preparation of 3-phytase produced by Komagataella phaffii CECT 13094 as a feed additive for pigs for fattening, minor porcine species, turkeys for fattening and reared for breeding (holder of authorisation: Fertinagro Biotech S.L.). OJ L 65, 25.2.2021, p. 43.
7 FEED dossier reference: FAD-2020-0001.
8 The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2017-0043_en.
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.
3.1. Characterisation

3.1.1. Characterisation of the production organism

The production strain is a genetically modified strain of *K. phaffii* which is deposited at the Spanish Type Culture Collection with the deposition number CECT 13171.10

The data submitted to support the taxonomic identification of the strain included two data sets. In the first,11 the whole genome of the production strain was sequenced, and the reads were either reference-based assembled or mapped against two reference strains, . The information provided did not allow to conclusively establish the taxonomic identification of the strain. The second data set included a phylogenomic analysis.12 This second analysis was done considering only the ITS sequence and no details were provided regarding the strains used for comparison in the analysis. Therefore, the two data sets were considered insufficient for the assessment. Consequently, the FEEDAP Panel cannot conclude on the taxonomic identification of the production strain.

3.1.1.1. Information related to the genetic modification

3.1.1.1.1. Characteristics of the parental and recipient strains

3.1.1.1.2. Genetic modification of the production strain

3.1.2. Characterisation of the additive

FSF10000 is the solid formulation of the additive and contains enzyme protein (1.2%), sodium citrate (3%-7%), wheat bran (88%-92%) and water (2%-8%). The FLF1000 formulation contains

10 Technical dossier/Section II/Annex II.16.
11 Technical dossier/Section II/Annex II.12.
12 Technical dossier/Supplementary information April 2022/Annex 1.
enzyme protein (1.2 g/L; range 1.1–1.3 g/L), sorbitol (28%–32%), sodium acetate trihydrate (0.4%–0.6%), potassium sorbate (0.08%–1.20%) and water (68%–72%). According to the applicant, the manufacturing process and the final formulations are the same as those described for the 3-phytase produced by K. phaffii CECT 13094 in previous assessments (EFSA FEEDAP Panel, 2016, 2019a). Therefore, the information/data regarding the manufacturing, composition and physicochemical properties of the 3-phytase produced by strain CECT 13094 assessed and described by the FEEDAP Panel, are considered to apply to the 3-phytase produced by K. phaffii CECT 13171. The applicant provided new data on the batch-to-batch variation and on the purity of the two formulations that are described below.

The FSF10000 formulation ensures a minimum of 10,000 FTU/g and the FLF1000 ensures a minimum of 1,000 FTU/mL. Data on the batch-to-batch variation of the enzyme activity were provided for five batches of each form of the additive. The mean phytase activity in the solid formulation was 10,672 FTU/g additive (range from 10,411 to 11,125 FTU/g) and in the liquid formulation was 1,070 FTU/mL (ranging 1,008 to 1,101 FTU/mL). These data show compliance with the specifications of the additive.

Data on the chemical and microbiological contaminants were provided for three batches of each form of the additive. The results showed Enterobacteriaceae < 4.0 × 10^4 colony forming units (CFU)/g in the solid and < 1 CFU/mL in the liquid, filamentous fungi ≤ 1.1 × 10^2 and 5.2 × 10^2 CFU/g or mL, yeasts < 4 × 10^4 or < 10 CFU/g or mL, and Salmonella absent in 25 g or mL. The contents of lead, cadmium, mercury, ochratoxin A, aflatoxins B1, B2, G1 and G2, deoxynivalenol, zearalenone, T-2 toxin, HT-2 toxin, fumonisins B1 and B2 were all below the respective limits of quantification.

The presence of viable cells was tested in three batches of the liquid and solid formulations in triplicate. Ten millilitres of the liquid formulation or 10 g of the solid product was diluted in 90 mL of sterile water. The filtrate was then centrifuged for 10 min at 5,000 g and the pellet suspended in 3 mL of YPD medium and incubated at 30°C for 4 h. Samples were then plated on yeast extract-peptone-dextrose agar and incubated for up to 10–15 days at 25°C. A positive control was included. No cells were detected in the samples tested. However, the information given was not clear as regards whether the positive control was the spiked the samples tested with the production strain. Therefore, uncertainty remains on whether the method would enable the growth of any possible viable cells remaining in the product. Consequently, the FEEDAP Panel is not in the position to exclude the presence of viable cells in the final additive.

The presence of recombinant DNA was analysed by PCR in 1 g samples of three batches of solid and liquid formulations, tested in triplicate. The DNA was extracted and purified by using a commercial kit and including a mechanical lysis step with glass beads and a beadbeater. Four genes were targeted with a limit of detection ranging from 0.1 to 10 ng/g or mL. Positive and negative controls were included. No recombinant DNA was detected in any of the solid and liquid batches tested.

### 3.1.3. Conditions of use

The additive is intended to be used in feed for poultry species for fattening or reared for fattening/breeding, pigs for fattening and minor porcine species for growing at 500 FTU/kg feed and in laying hens at 375 FTU/kg feed. The maximum recommended level is 1,000 FTU/kg.

### 3.2. Safety

#### 3.2.1. Safety of the production strain

The recipient strain from which the production organism was derived belongs to K. phaffii, which is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety
assessment when used for enzyme production (EFSA BIOHAZ Panel, 2007, 2020). To apply the QPS approach to safety assessment, the strain should be unambiguously identified, the genetic modification be of no concern and no production strain be in the final product. The applicant provided information showing that the genetic modifications present in the production strain do not raise safety concerns and that no recombinant DNA of the production strain was detected in the final additive. However, the data provided did not allow to unambiguously identify the production strain and uncertainties remain regarding the possible presence of viable cells of the production strain in the final product. Therefore, the FEEDAP Panel cannot conclude on the safety of the production strain *K. phaffii* CECT 13171.

### 3.2.2. Safety for the target species

In previous opinions, the FEEDAP Panel concluded on the safety for the target species of the 3-phytase produced by *K. phaffii* CECT 13094. The Panel concluded based on studies conducted in chickens for fattening, laying hens and pigs for fattening that the additive is safe for poultry species for fattening or reared for laying/breeding, laying hens, pigs for fattening and minor porcine species at the maximum recommended level of 1,000 FTU/kg (EFSA FEEDAP Panel, 2016, 2018a, 2019a,b, 2020a,b).

The 3-phytase gene present in the strain under evaluation, *K. phaffii* CECT 13171, is the same as the one present in strain CECT 13094 and the final formulations of the additive are the same. Moreover, the maximum level proposed for the target species has not been modified. Therefore, the Panel considers that the conclusions reached in studies conducted with the 3-phytase produced with CECT 13094 apply to the 3-phytase obtained with *K. phaffii* CECT 13171.

However, considering the uncertainties in the taxonomic identification of the strain and the potential presence of viable cells in the final formulations, the Panel cannot conclude on the safety of the additive for the target species.

### 3.2.3. Safety for the consumers

No toxicological studies have been submitted by the applicant to support the safety of the additive for the consumers since it was considered that the safety of the product would be presumed considering the fact that the production strain belongs to a species considered for the QPS approach. However, the information/data submitted by the applicant regarding the taxonomic identification of the production strain and the possible presence of viable cells do not allow to unambiguously identify the production strain, or to conclude on the absence of viable cells in the final product. Therefore, the safety of the additive cannot be presumed, and consequently, the FEEDAP Panel cannot conclude on the safety for the consumer of the additive.

### 3.2.4. Safety for the users

In previous assessments, the Panel evaluated the safety for the user of the 3-phytase produced by *K. phaffii* CECT 13094 (EFSA FEEDAP Panel, 2016, 2019a). The studies assessed included skin and eye irritation, and dermal sensitisation which were conducted with the solid and the liquid formulations of the additive. The FEEDAP Panel concluded that the additive in either form is not irritant to eyes and skin; that the liquid formulation is not a dermal sensitisier but the solid formulation is, and the two formulations should be considered potential respiratory sensitisers.

No new information has been provided. However, the 3-phytase gene in the product under evaluation is the same as the one previously evaluated, and the formulated additives share the same composition. Therefore, the FEEDAP Panel considers that the conclusions reached regarding the irritancy and sensitisation potential would apply for the additive under assessment.

However, considering the uncertainties in the identification of the strain and the possible presence of viable cells in the final formulations, the Panel cannot conclude on the safety of the additive for users.

### 3.2.5. Safety for the environment

The active substance of the additive is a protein, and as such will be degraded/inactivated during passage through the digestive tract of animals or in the environment; therefore, no concerns would arise from the 3-phytase present in the additive.

The genetic modification of the production strain raises no safety concerns, and no recombinant DNA was detected in the final formulations of the additive, but uncertainty remains as regards to the identification of the production strain and the presence of viable cells in the product.
3.3. Efficacy

In previous opinions, the FEEDAP Panel evaluated the efficacy of the 3-phytase produced by K. phaffii CECT 13094. At this respect, the Panel concluded based on studies conducted in chickens for fattening, laying hens and pigs for fattening that the additive is efficacious at 500 FTU/kg feed for poultry species for fattening or reared for laying/breeding, pigs for fattening and minor porcine species, and at 1,000 FTU/kg feed in laying hens (EFSA FEEDAP Panel, 2016, 2018a, 2019a,b, 2020a). The 3-phytase gene present in the strain under evaluation, K. phaffii CECT 13171, is the same as the one present in strain CECT 13094. Therefore, the Panel considers that the conclusions reached in studies conducted with the 3-phytase obtained with CECT 13094 apply to the 3-phytase obtained with K. phaffii CECT 13171.

No new information has been provided that would lead the Panel to reconsider the conclusions previously drawn in poultry species for fattening or reared for laying/breeding, pigs for fattening and minor porcine species for fattening. However, the applicant is proposing a new use level (375 FTU/kg feed) in laying hens which is lower than the authorized, and provided a new study to support the efficacy, which is described below.

3.3.1. Efficacy for laying hens

In 2016, the FEEDAP Panel considered three trials in the assessment: two short-term trials and one long-term trial. The two short-term trials showed a significant increase in the phosphorus retention in the birds receiving the 3-phytase, one at 1,000 FTU/kg feed and the other one at 250 FTU/kg feed. The results of the long-term trial showed an improvement on the daily egg mass production from 250 FTU/kg diet.

The applicant submitted another short-term trial aimed at studying the effect of the additive on the ileal digestibility and bone mineralisation. A total of 108 25-week-old laying hens (Hy-line) were randomly caged in groups of three animals and allocated to two dietary treatments (representing 12 replicates in the control and 24 in treated group). For 9 days, all hens received the same diet (4.9% calcium and 0.54% of phosphorus). After these 9 days, the hens were fed a basal diet based on maize and soybean meal, with a total content of phosphorus of 0.42% and calcium of 3.33%, which was either not supplemented (control) or supplemented with the 3-phytase to provide 375 FTU/kg feed (FSF10000 obtained from CECT 13094). The phytase activity was measured in the control and supplemented diets prior the start of the study and at the end, and values found were 0 for the control and 390 FTU/kg feed for the supplemented. Health status of the animals was monitored daily. The diets were administered in mash form on ad libitum basis for 14 days and presented an external marker for the digestibility measurements. The hens were weighed in groups on days 0 and 14 under study, feed intake was measured per cage for the same period. Total number of eggs produced, and egg weight were recorded, and egg mass and feed to egg ratio were calculated. On the last day of the study, all the hens were killed, ileal contents and left tibia bones were collected and pooled per cage. Ileal contents were used to determine the digestibility of phosphorus. Bones were dried, weighed and ash/phosphorus content was determined. All eggs laid in the last 24 h of the study were weighed and freeze-dried for phosphorus content analysis. An analysis of variance was done with the data considering the treatment as the effect, the pen/cage being the experimental unit. Initial body weight was used as covariate for the performance parameters. Group means were compared with Tukey test.

The results (Table 1) showed an improvement on the ileal digestibility of phosphorus in the hens fed the 3-phytase compared to control. The hens fed the 3-phytase showed a higher relative tibia weight (percent of body weight) and a higher total ash and phosphorus content in the tibia (g/tibia; data not shown) compared to the control. However, in order to conclude on the efficacy, improvements on the relative mineralisation in the tibia should be observed concomitantly to the improvements on the ileal digestibility. The relative amount of ash and phosphorus in tibia was not different between the two groups and consequently, the Panel considers that no conclusion can be drawn as regards to the effect of the 3-phytase on the relative retention.

Therefore, the newly submitted data do not allow the Panel to conclude at the newly proposed level of 375 FTU/kg feed.
3.3.2. Conclusions on the efficacy

Based on the studies provided for the 3-phytase and those previously evaluated, the Panel concludes that the 3-phytase present in the additive under evaluation is efficacious at 500 FTU/kg feed in poultry species for fattening or reared for laying/breeding, pigs for fattening and minor porcine species and at 1,000 FTU/kg feed in laying hens.

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\(^\text{20}\) and Good Manufacturing Practice.

4. Conclusions

The genetic modifications present in *K. phaffii* CECT 13171 do not raise safety concerns and no recombinant DNA was detected in the final formulations of the additive. However, the Panel cannot conclude on the identity of the production strain and uncertainty remains on the possible presence of viable cells of the production strain in the final formulations. Owing to these uncertainties, the FEEDAP Panel cannot conclude on the safety of the additive regarding the production strain.

The enzyme 3-phytase present in the additive is considered safe for poultry species for fattening or reared for laying/breeding, laying hens, pigs for fattening and minor porcine species at the maximum recommended level of 1,000 FTU/kg feed. The additive in either form is not considered irritant to eyes and skin. The liquid formulation is not considered a dermal sensitiser but the solid formulation is, and the two formulations should be considered potential respiratory sensitisers.

The 3-phytase present in the additive does not raise safety concerns for the environment.

Considering the uncertainties on the identification of the production strain and the possible presence of viable cells in the final formulations, the Panel cannot conclude on the safety of the additive for the target species, consumer, users, and the environment.

The FEEDAP Panel concludes that the additive is efficacious at 500 FTU/kg feed in poultry species for fattening or reared for laying/breeding, pigs for fattening and minor porcine species and at 1,000 FTU/kg feed in laying hens.

5. Documentation provided to EFSA/Chronology

| Date       | Event                                                                 |
|------------|----------------------------------------------------------------------|
| 03/01/2020 | Dossier received by EFSA. 3-phytase for poultry and pigs. Submitted by Fertinagro Biotech S.L. |
| 22/01/2020 | Reception mandate from the European Commission                        |
| 04/06/2021 | Application validated by EFSA – Start of the scientific assessment   |
| 06/07/2021 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation* |
| 06/09/2021 | Reception of supplementary information from the applicant – Scientific assessment re-started |
| 16/09/2021 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation* |

\(^{20}\) 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.

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**Table 1:** Effects of 3-phytase on the ileal apparent digestibility of phosphorus, egg phosphorus content and tibia parameters

| Groups (FTU/kg feed) | Ileal digestibility of P (%) | Phosphorus egg content (%) | Tibia Ash (% of DM) | Phosphorus (% of ash) |
|----------------------|-----------------------------|---------------------------|---------------------|-----------------------|
| 0                    | 25.2\(^a\)                  | 0.54                      | 44.3                | 17.5                  |
| 375                  | 36.5\(^a\)                  | 0.53                      | 44.9                | 17.4                  |

\(^{a,b}\)Mean values within a column with different superscript are different (p < 0.05).
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**Abbreviations**

- **CFU**: colony forming unit
- **EURL**: European Union Reference Laboratory
- **FEEDAP**: EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed