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Safety and efficacy of APSA PHYTAFEED® 20,000 GR/L (6-phytase) as a feed additive for chickens for fattening, chickens reared for laying and minor growing poultry species

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Abstract

APSA PHYTAFEED® 20,000 GR/L is a preparation of 6-phytase which is presented in solid and liquid forms. This additive is intended to be used as a zootechnical additive in chickens for fattening or reared for laying/breeding and minor poultry species for fattening or reared for laying/breeding. The 6-phytase present in the additive is produced by a genetically modified strain of *Komagataella phaffii*. The production strain and its recombinant DNA were not detected in intermediate products used to produce the additive. The final products do not trigger a safety concern with regard to the genetic modification. Based on the results obtained in a tolerance study in chickens for fattening and the data from a subchronic oral toxicity study the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the additive is safe for chickens for fattening. This conclusion was extended to chickens reared for laying/breeding and extrapolated to all minor poultry species for fattening or reared for laying/breeding. The FEEDAP Panel concluded that the use of APSA PHYTAFEED® 20,000 GR/L as a feed additive gives rise to no concern for consumers. The additive, in either form, is not toxic by inhalation or irritant for skin or eyes and it is not a dermal sensitizer, but it is considered a potential respiratory sensitizer. The use of the product as a feed additive is of no concern for the environment. The FEEDAP Panel evaluated three efficacy trials in which the retention of the phosphorus was studied. The data showed that the additive has the potential to improve the retention of phosphorus in the diets in chickens for fattening at 250 U/kg feed. This conclusion was extended to chickens reared for laying/breeding and extrapolated to all minor poultry species for fattening or reared for laying/breeding.

Keywords: zootechnical additives, digestibility enhancers, phytase, safety, efficacy, chickens

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Summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the safety and efficacy of APSA PHYTAFEED® 20,000 GR/L (6-phytase). This additive is a preparation of 6-phytase and is presented in solid and liquid forms, and it is intended to be used as a zootechnical additive (digestibility enhancer) in chickens for fattening or reared for laying/breeding and minor poultry species for fattening or reared for laying/breeding.

The phytase present in the additive is produced by a genetically modified strain of *Komagataella phaffii*. The production strain and its recombinant DNA was not detected in the intermediate products used to formulate the additive. The final products do not trigger a safety concern with regard to the genetic modification.

The solid and the liquid formulations are considered equivalent in terms of safety and efficacy for the target species.

The recipient strain belongs to a species which is considered to qualify for the Qualified Presumption of Safety (QPS) approach to safety assessment and the genetic modification to which it was subject to does not give rise to concern. The results from toxicological studies including genotoxicity and a subchronic oral toxicity study supported this conclusion. Therefore, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the use of APSA PHYTAFEED® 20,000 GR/L as a feed additive gives rise to no concern for consumers.

Based on the results obtained in a tolerance study in chickens for fattening and the data from a subchronic oral toxicity study the FEEDAP Panel concluded that the additive is safe for chickens for fattening. This conclusion was extended to chickens reared for laying/breeding and extrapolated to all minor poultry species for fattening or reared for laying/breeding.

The additive, in either form, is not toxic by inhalation or irritant for skin or eyes and it is not a dermal sensitizer, but it is considered a potential respiratory sensitizer.

The use of the product as a feed additive is of no concern for the environment.

The FEEDAP Panel evaluated three efficacy trials in which the retention of phosphorus was studied. The data showed that the additive has the potential to improve the retention of phosphorus in the diets in chickens for fattening at 250 U/kg feed. This conclusion was extended to chickens reared for laying/breeding and extrapolated to all minor poultry species for fattening or reared for laying/breeding.

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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 Andrés Pintaluba S.A.[2] for authorisation of the product APSA PHYTAFEED® 20,000 GR/L (6-phytase), when used as a feed additive for chickens for fattening, chickens reared for laying and minor poultry species (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 18 July 2018.

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 APSA PHYTAFEED® 20,000 GR/L (6-phytase), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

APSA PHYTAFEED® 20,000 GR/L is a feed additive that contains 6-phytase to be used in feed for chickens for fattening, chickens reared for laying and minor poultry species as a zootechnical additive. It has not been previously authorised in the European Union.

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[3] in support of the authorisation request for the use of APSA PHYTAFEED® 20,000 GR/L as a feed additive.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL report can be found in Annex A.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of APSA PHYTAFEED® 20,000 GR/L is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c), Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008b), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP

[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] Andrés Pintaluba S.A. Pol. Ind. Agro Reus, c/. Prudenci Bertrana, 5, Reus 43206, Spain.
[3] FEED dossier reference: FAD-2018-0031.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports
[5] 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.

Panel 2012d), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017) and Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011).

3. Assessment

The additive APSA PHYTAFEED® 20,000 GR/L, available in solid and liquid forms, contains 6-phytase (EC 3.1.3.26; phytase) and it is intended to be used in feed for chickens for fattening, chickens reared for laying and minor poultry species as a zootechnical additive (functional group: digestibility enhancers).

3.1. Characterisation

3.1.1. Characterisation of the active substance

The phytase present in the additive is produced by a genetically modified strain of the yeast *Komagataella phaffii* that has been deposited in the China General microbiological Culture Collection Centre (CGMCC) with the deposit number 12056.[6] [redacted]

3.1.1.1. Information relating to the genetic modification

Characteristics of the recipient strain

[redacted]

3.1.1.2. Characteristics of the donor organism

[redacted]

3.1.1.3. Description of the genetic modification process

[redacted]

3.1.2. Manufacturing process

The enzyme is produced by fermentation with the production strain. The product obtained after fermentation is filtrated and concentrated [redacted]

[6] Technical dossier/Section II/Annex II.2.1.2.Conf. [redacted]

[redacted]

3.1.3. Characterisation of the additive

The two formulations of the additive ensure a guaranteed minimum phytase activity of 20,000 U[12]/g or mL of product.

The solid formulation, APSA PHYTAFEED® 20,000 GR, [redacted]. The batch-to-batch variation of this formulation was studied in five batches and the mean value was 23,370 U/g product, ranging from 21,600 to 24,700 U/g (with a coefficient of variation (CV) of 5.6%).[13] The mean particle size measured in three batches by laser diffraction was 227–243 μm, with no particles below 10 μm and less than 1% of particles below 50 μm.[14] The dusting potential measured in three batches by the Stauber–Heubach method was ≤ 30 mg/m^3.[15] The bulk density of the product is of 620 kg/m^3.[16]

The liquid formulation, APSA PHYTAFEED® 20,000 L, [redacted]. The batch-to-batch variation of this formulation was studied in five batches and the mean value was 23,188 U/ml, ranging from 21,800 to 24,600 U/ml (CV of 4.3%).[13] This form of the additive has a viscosity similar to 7 mPa·s at 20°C and a surface tension of 44 dyn/cm^2.[17]

The two formulations were analysed for chemical and microbiological contamination (at least three batches in each analysis).[18] The analysis of the chemical contamination included arsenic [redacted], cadmium [redacted], lead [redacted], mercury [redacted], fluorine [redacted], aflatoxin B1 [redacted] deoxynivalenol [redacted], zearalenone [redacted] fumonisin B1 [redacted] fumonisin B2 [redacted] ochratoxin A [redacted] toxin T-2 and toxin HT2 [redacted] methanol [redacted] and the sum of dioxins and dioxin-like PCBs [redacted]. Microbial analysis included total coliform bacteria [redacted], *E. coli* [redacted] and *Salmonella* spp. [redacted]. No antimicrobial activity was detected in three batches of the enzyme product [redacted] Therefore, the production strain and its DNA were not detected in the intermediate products [redacted]. These results apply to the final product [redacted].

[redacted]

[12] Unit, one unit 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.
[13] Technical dossier/Section II/Annex II.1.3.3.
[14] Technical dossier/Section II/Annex II.1.5.1.
[15] Technical dossier/Section II/Annex II.1.5.2.
[16] Technical dossier/Section II/Annex II.1.5.3.
[17] Technical dossier/Section II/Annex II.1.5.4.
[18] Technical dossier/Section II/Annex II.1.4.1.3, II.1.4.1.4, II.1.4.1.5.1 and II.1.4.1.5.2 and supplementary information February 2019.

[redacted]

3.1.4. Stability and homogeneity

The shelf-life of the additive is claimed to be 12 months for the solid formulation and 6 for the liquid when stored at 20–25°C in closed packages. The shelf-life of the two formulations was studied in three batches of either formulation stored in the closed containers at different temperatures and time points.[21] The enzyme activity in the solid formulation was 85% of the initial activity (25,500 U/g) when stored at 25°C for 12 months and 40% of the initial activity when stored at 40°C for 6 months. The enzyme activity in the liquid formulation was 95% of the initial activity (23,750 U/g) when stored at 5°C for 12 months (86% after 24 months under the same conditions) and 85% of the initial when stored at 20°C for 6 months.

The stability of the phytase in a vitamin and mineral premixture (without choline chloride) was studied in three batches of the solid formulation added to provide 125,000 U/kg premixture.[22] Samples were stored for 6 months at 25°C (container not specified). The enzyme activity after 6 months was 84% of the initial enzyme activity.

The stability of the phytase in feed was evaluated for the two formulations (one batch each) when added to a complete feed for chickens for fattening.[23] The two formulations of the additive were added to a mash feed to provide 1,000 U/kg feed, the mash feed supplemented with the solid formulation was also granulated in order to study the effect of the temperature. Recovery values after granulation showed no modifications of the initial enzyme activity (recovery of 100%). Samples of the mash and granulated were stored for 3 months at 25°C (containers not specified). After 3 months of storage, recovery values showed no modifications of the initial enzyme activity.

The capacity of the phytase to homogeneously distribute was studied in 10 subsamples of the premixture and feeds used in the stability studies. Samples analysed for the premixture showed a CV of 9.6%. Samples of the mash feeds showed a CV of 8% for the solid formulation or of 6% for the liquid formulation and the samples of the pelleted feed showed a CV of 6%.

3.1.5. Conditions of use

The additive is intended to be used in feed for chickens for fattening, chickens reared for laying and minor poultry species for fattening purposes or up to the point of lay at a minimum enzyme activity of 250 U/kg feed.

3.2. Safety

3.2.1. Safety of the genetic modification

The recipient strain from which the production organism was derived belongs to *K. phaffii*, which is considered by EFSA to be suitable for the QPS approach to safety assessment when used for enzyme production (EFSA BIOHAZ Panel, 2017).

[redacted] The production strain was identified as *K. phaffii* and the traits introduced raise no safety concerns. Therefore, the additive does not pose any safety concern with regard to the genetic modification of the production strain.

3.2.2. Toxicological studies

Toxicological studies are not required for fermentation products produced by a genetically modified microorganism for which the recipient strain is considered by EFSA to qualify for the QPS approach to safety assessment and for which the genetic modification raises no concerns. However, the applicant submitted the below studies to support the safety of the additive.

Bacterial reverse mutation test

The liquid formulation of the additive was tested for the induction of reverse mutations in *Salmonella* Typhimurium tester strains TA1535, TA1537 TA98, TA100 and TA102.[24] The experimental

[21] Technical dossier/Section II/Annex II.4.1.1.1 and II.4.1.1.2.
[22] Technical dossier/Section II/Annex II.4.1.2.
[23] Technical dossier/Section II/Annex II.4.1.3.1 and II.4.1.3.2.
[24] Technical dossier/Section III/Annex III.2.2.2.1.

protocol was in line with Organisation for Economic Co-operation and Development (OECD) guideline 471. The test item was dissolved in distilled water and tested both in the presence and absence of the metabolic activation system at five different concentration levels up to 100 μL/plate[25] in two independent studies. In the second test, the pre-incubation method was used in the presence of metabolic activation system.

No significant toxicity was observed but significantly increased numbers of revertant colonies were observed in the positive controls. Statistically significant increases in the number of revertant colonies were reported in strain TA1535 and TA102, in the absence of metabolic activation. The Panel noted that the fold increase in revertant colony numbers was always below 2, the values were within the historical vehicle control ranges and most of the results were not reproducible; on this basis, the observed increases were considered not biologically relevant. It was concluded that the test item did not induce gene mutations in bacteria under the test conditions employed in this study.

In vitro *mammalian cell micronucleus test*

The liquid formulation of the additive was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy. The experimental protocol was in line with OECD guideline 487.[26] The maximum tested concentrations was established in a preliminary experiment showing the induction of osmolarity changes (> 50 mOsmol compared to the culture medium) at concentrations from 2.5% test item and above. Cells were stimulated for 44–48 h with phytohaemaglutinin and then treated for 4 h (followed by a 24-h recovery period) with concentrations ranging from 0.2 to 12.5 μL/mL dissolved in distilled water both in the absence and in the presence of S9-mix. In a parallel assay, cells were treated for 28 h with the same doses of the test item in the absence of S9-mix with no recovery period. The positive controls induced statistically significant increases in the frequency of micronuclei, demonstrating the sensitivity of the test system and the efficacy of the S9-mix. No significant cytotoxicity was observed. No statistically significant increase in the frequency of micronuclei was observed at any dose and sampling time. It was concluded that the test item did not induce micronuclei in cultured human peripheral blood lymphocytes under the experimental conditions employed in this study.

Subchronic oral toxicity study

Sprague–Dawley CRl:OFA rats (10/sex per group) received the fermentation product by oral gavage at 0 (control – water), 29,807, 59,614 or 119,228 U/kg body weight (bw) per day for 90 consecutive days (up to 2 mL/kg bw per day). The study was conducted in compliance with OECD guideline 408 (1998).

In total, four rats died. One female in the highest dose died right after the first dosing (and was replaced by a spare one). Two males (one from 29,807 and another one from 119,228 U/kg bw per day groups) and one female (control group) died due to gavage practice.

Some clinical signs were noted on the males receiving the test item but these were isolated and not treatment related. No differences were found on the food intake or in the body weight of the animals. No abnormalities were identified in the ophthalmological examinations conducted. No changes in the urinalyses, blood chemistry parameter or in the haematological and coagulation parameters were found with the exception of a slightly lower activated partial thromboplastin time in females in the 29,807 U/kg bw group. No differences were found on the organ's weight and the macroscopic examination revealed similar incidence of isolated changes in the control and the dose groups. The histopathological lesions noted were deemed to be incidental and not treatment related.

On the basis of the study, the Panel identified the no observed adverse effect level (NOAEL) of 119,228 U/kg bw per day (the highest dose tested).

3.2.3. Safety for the target species

In order to support the safety of the additive for the target species, the applicant submitted a tolerance study and made reference to the results obtained in the subchronic oral toxicity study presented in Section 3.2.2.

A total of 192 one-day-old male chickens for fattening (Ross 308) were distributed in 48 cages of 4 birds.[27] Six dietary treatments were allocated to the pens, representing eight replicates per treatment. Two basal diets (starter and grower) based on maize and soya bean meal (total phosphorus 4.0 and

[25] Dose established in a preliminary toxicity assay.
[26] Technical dossier/Section III/Annex III.2.2.2.2.
[27] Technical dossier/Section III/Annex III.1.1.1 and Supplementary information February 2019.

3.9 g/kg, total calcium 7.2 and 6.2 g/kg, respectively) were either not supplemented (control) or supplemented with APSA PHYTAFEED® 20,000 GR to provide 250 (1× recommended level), 500, 1,000 or 100,000 (400× recommended level) U per kg feed (confirmed by analysis). A positive control diet with higher content of phosphorus was also considered (total phosphorus 5.9 g/kg for starter and grower diets and total calcium 7.1 and 6.1 g/kg for starter and grower diets, respectively). Diets were offered in mash form for 35 days. Mortality and health status were checked every day and dead animals were necropsied. Animals were weighed on days 0, 21 and 35 (cage basis), feed intake was registered throughout the study per cage and feed to gain ratio was calculated. Blood samples were obtained from 1 bird per cage on day 35 for haematology and blood biochemistry.[28] An analysis of variance (ANOVA) was done with the data and considering the treatment as a fixed effect and block (situation of the cage) as a random effect. Group means were compared with the Tukey test. The significance level was set at 0.05.

Mortality including culling was 4% and not treatment related. The results on the feed intake, body weight and feed to gain ratio are presented in Table 1. Birds in the control diet showed a statistically significant lower feed intake and final body weight compared to the other groups. The body weight gain in the control group was 30% lower compared to performance objectives for the breed. No differences in the feed intake, final body weight and feed to gain ratio of the birds were found between the groups fed the phytase and the positive control, with the exception of a lower feed intake in the 250 U/kg feed compared to positive control. No statistical differences were observed in any of the parameters measured in blood.

Table 1: Effect of APSA PHYTAFEED® 20,000 on the performance of chickens for fattening

Groups (U/kg feed)	Daily feed intake (g)	Final body weight (g)	Feed to gain ratio	Mortality (n)
0	65.1^{c}	1,563^{c}	1.50	3
250	75.0^{b}	1,820^{b}	1.48	1
500	80.6^{ab}	1,913^{ab}	1.51	1
1,000	81.5^{ab}	1,974^{ab}	1.48	1
100,000	83.4^{a}	2,040^{a}	1.47	0
Positive control	82.8^{a}	1,970^{ab}	1.51	2

^{a,b,c}values in the same column not sharing the same superscript are significantly different ($p < 0.05$).

The low performance registered in the control group does not allow to use it as a control to identify any impairment of the performance in animals receiving the phytase. However, the data from the groups receiving the phytase did not show a negative dose-related trend with increasing levels of the phytase in the performance; in fact, improvements on the performance were seen with increasing levels of the phytase. Moreover, no significant differences in the performance of the birds that received the phytase were evidenced compared to the positive control diet. Therefore, the data from this tolerance trial would indicate that the birds could tolerate well the phytase at the recommended level.

A subchronic oral toxicity study was provided in support to the safety for the target species (described in Section 3.2.2). The results of that study indicate a NOAEL of 119,228 U/kg bw in rats. From this NOAEL, the applicant calculated the maximum safe level for chickens for fattening in feed according to the guidance on the safety for the target species (EFSA FEEDAP Panel, 2017). The result of the calculation was 13,281 U/kg feed, and this would support the results of the tolerance study and indicate a wide margin of safety, approximately 50.

3.2.3.1. Conclusions on the safety for the target species

The FEEDAP Panel concludes that APSA PHYTAFEED® GR/L is safe for chickens for fattening at the recommended level of 250 U/kg feed with a wide margin of safety. This conclusion is extended to chickens reared for laying and considering the wide margin of safety, the conclusion can be extrapolated to minor poultry species for fattening purposes or reared for laying/breeding purposes. The solid and the liquid formulations are considered equivalent in terms of safety for the target species.

[28] Total count for red blood cells, packed cell volume, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total and differential counts for leukocytes, total protein, albumin, uric acid, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase.

3.2.4. Safety for the consumer

The enzyme is produced by a genetically modified strain of *K. phaffii*; this species is considered to qualify for the QPS approach to safety assessment when used for enzyme production. The identity of the strain was established, and the genetic modification of the production strain raises no concerns. Therefore, the production strain is presumed safe for production purposes and no concerns would raise for the consumer from the fermentation product obtained from this strain. The results obtained in the genotoxicity studies and the subchronic oral toxicity support this conclusion.

3.2.5. Safety for the user

3.2.5.1. Effects on the respiratory system

No specific tests were submitted; however, based on the proteinaceous nature of the active substance of the additive, it is considered as a potential respiratory sensitiser.

3.2.5.2. Effects on the skin and eyes

The skin and eye irritation potential of the liquid and the solid formulations of the additive were tested in valid studies performed according to OECD guideline 404 and 405, which showed that they are not irritants to skin or eyes.[29]

Both solid and liquid formulations were tested for skin sensitisation following the OECD guideline 406.[30] The solid formulation tested at 100% concentration elicited at 24 h and 48 h, in two and one animals (corresponding to 20% and 10% of animals tested, respectively) a slight erythema. The liquid formulation tested at 100% concentration elicited in two animals (20% of animals tested) mild sensitisation after 24 and 48 h. According to the classification, labelling and packaging of substances and mixtures criteria (European Chemical Agency, 2017), the two formulations are classified as non-dermal sensitizers.

3.2.5.3. Conclusions on safety for the user

The additive, in either form, is not a skin or eye irritant and it is not a dermal sensitizer. However, owing to the proteinaceous nature of the active substance, it should be considered a potential respiratory sensitizer.

3.2.6. Safety for the environment

The production strain and its DNA were not detected [redacted]. The additive does not raise safety concerns for the environment with regard to the genetic modification of the production strain.

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 risks to the environment are expected and no further environmental risk assessment is required.

3.3. Efficacy

Two balance trials and a performance trial, which included a balance, done in chickens for fattening were submitted for the assessment.

The two short-term trials shared a similar design. In the first, 320 one-day-old male chickens for fattening (Arbor Acres plus) were distributed in 40 cages in groups of 8 birds.[31] In the second, 480 one-day-old male chickens for fattening (Cobb 500) were distributed in 24 pens in groups of 20 birds.[32] Basal diets based on maize and soya bean meal were either not supplemented (control) or supplemented with APSA PHYTAFEED® 20,000 GR to provide different levels of the phytase including the recommended level in the two studies (Table 2, trials 1 and 2). The enzyme activities were confirmed by analysis. In trial 1, a positive control diet with higher content of phosphorus was also considered. Diets were offered in mash form for 21 or 28 days, respectively, and contained titanium dioxide as an external marker. Mortality and health status were checked every day and dead animals were necropsied. Animals were weighed on days 0 and 21 (cage basis) in trial 1 or on weekly basis in

[29] Technical dossier/Section III/Annexes III.3.1.2.1 to III.3.1.2.4.
[30] Technical dossier/Section III/Annexes III.3.1.2.5 and III.3.1.2.6.
[31] Technical dossier/Section IV/Annex IV.2.1 and Supplementary information IV.2.1.
[32] Technical dossier/Section IV/Annex IV.2.2.

trial 2. Feed intake was measured throughout the study period. In trial 1, the balance study was conducted on days 19–21 of life, with total collection of excreta for 4 days. In trial 2, on day 26 of life, two birds per pen were selected based on average body weight of the pen and were individually caged (16 birds per treatment) to perform the balance study, excreta samples were collected for three days (days 26–28). At the end of the balance trials, one bird per cage in trial 1 or two birds per cage in trial 2 were killed and tibia bones were collected. Feed and excreta samples were analysed for titanium, phosphorus, calcium, dry matter and ash to determine the utilisation and tibia content of ash (trial 1) or ash and phosphorus (trial 2). An ANOVA was done with the data and group means were compared with the Tukey test. The significance level was set at 0.05.

The long-term trial is the tolerance trial presented in Section 3.2.1. In that trial, the performance of the birds was measured and a balance study was performed on days 19–21 of life of the chicks. The diets contained titanium dioxide as an external marker and the total excreta was collected during the study period. Feed and excreta samples were analysed for the marker, ash, dry matter, calcium and phosphorus to determine the utilisation. Moreover, one bird per cage, randomly chosen on day 21, was killed for tibia bones collection. The bones were analysed for ash content.

The results of the balance trials are presented in Table 2 and the performance parameters of trial 3 in Table 1 (see Section 3.2.2).

Table 2: Effect of APSA PHYTAFEED 20,000® on the phosphorus utilisation and tibia bone content[1]

Trial	Diets		Phosphorus retention (%)	Bone content (%)	
	Phytase (Units/kg feed)	Total P – Ca (g/kg feed)[2]		Ash	Phosphorus
1	0	5.7–9.0	46.5[b]	45.4[a]	–
	250	5.7–9.0	54.0[c]	51.2[b]	–
	500	5.7–9.0	55.2[c]	51.9[b]	–
	1,000	5.7–9.0	59.7[c]	52.9[b]	–
2	0	6.7–10.0	37.9[a]	52.4[b]	–
	0	3.7–8.4	37.6[a]	23.9[a]	3.9[a]
	250	3.7–8.4	47.8[b]	27.8[b]	4.9[b]
	500	3.7–8.4	52.3[c]	30.5[c]	5.5[c]
3	0	4.0–7.2	31.0[e]	36.4[b]	–
	250	4.0–7.2	43.9[c]	37.5[b]	–
	500	4.0–7.2	47.9[c]	38.5[b]	–
	1,000	4.0–7.2	61.3[b]	44.1[a]	–
	100,000	4.0–7.2	68.6[a]	46.8[a]	–
	Positive control	5.9–7.1	36.3[d]	43.3[a]	–

[1]Values are obtained from eight replicates per treatment in trials 1 and 3 and 16 in trial 2.
[2]Intended values for the diets administered during the balance trial.
[a,b,c,d,e]Values in the same column not sharing the same superscript are significantly different ($p < 0.05$).

The birds that received the phytase showed significant improvements on the phosphorus retention in the three balance trials submitted, the effects being seen from the addition of 250 U/kg feed. Also, improvements on the tibia mineralisation were observed in the three trials, in two trials from 250 U/kg feed and in one from 1,000 U/kg feed. These results would support the efficacy of the phytase in improving the utilisation of phosphorus in chickens for fattening from the addition of 250 U/kg feed.

In the long-term trial, the performance of the birds was improved by the phytase compared to control from the addition of 250 U/kg. The growth of the animals in the control group was rather slow, probably due to the low level of phosphorus in the control diet. However, a positive control was included in the study and the performance of the birds in this positive control group was not significantly different to the phytase groups. This result would also support the efficacy of the additive in improving the utilisation of phosphorus.

3.3.1. Conclusions on the efficacy

Based on improvements on the utilisation of phosphorus from three balance studies, the Panel concludes that APSA PHYTAFEED® 20,000 GR/L has the potential to be efficacious as a

zootechnical additive in chickens for fattening at the recommended level of 250 U/kg feed. This conclusion is extended to chickens reared for laying/breeding purposes. Considering that the mode of action of the phytases is well known and it is reasonably assumed to be the same among poultry species, the Panel extrapolates the conclusions on the efficacy to minor poultry species for growing or reared for laying/breeding. The solid and the liquid formulations are considered equivalent in terms of efficacy for the target species.

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[33] and Good Manufacturing Practice.

4. Conclusions

The production strain is considered safe for production purposes and the genetic modification raises no concerns. Viable cells of the production strain and its DNA were not detected in the additive.

APSA PHYTAFEED® 20,000 GR/L is safe for chickens for fattening or reared for laying/breeding and for minor poultry species for fattening or reared for laying/breeding at the recommended level of 250 U/kg feed.

The additive is safe for the consumers of food derived from animals fed with the additive.

The additive is not irritant for skin or eye and it is not a dermal sensitiser, but it is considered a potential respiratory sensitiser.

The use of the product as a feed additive is of no concern for the environment.

APSA PHYTAFEED® 20,000 GR/L has the potential to improve the utilisation of phosphorus in the diets in chickens for fattening or reared for laying/breeding and for minor poultry species for fattening or reared for laying/breeding at 250 U/kg feed.

Documentation provided to EFSA/Chronology

Date	Event
23/05/2018	Dossier received by EFSA. APSA PHYTAFEED® 20,000 GR/L. Andrés Pintaluba S.A.
06/06/2018	Reception mandate from the European Commission
18/07/2018	Application validated by EFSA – Start of the scientific assessment
25/09/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Control methods*
21/12/2018	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, safety for target species, and efficacy*
18/10/2018	Comments received from Member States
15/01/2019	Clarification teleconference during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products"
16/01/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
14/02/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
02/04/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ANOVA	analysis of variance
Bw	body weight
CFU	colony forming unit
CGMCC	China General microbiological Culture Collection Centre
CV	coefficient of variation
ECHA	European Chemicals Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
QPS	Qualified Presumption of Safety
PCB	polychlorinated biphenyls
PCDD/F	polychlorinated dibenzo-*p*-dioxins and dibenzofurans
TEQ	toxic equivalent
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for APSA PHYTAFEED® 20,000 GR/L

In the current application, authorisation in is sought under article 4(1) of Regulation (EC) No 1831/2003 for APSA PHYTAFEED® under the category/functional group 4(a) "zootechnical additives"/"digestibility enhancers". Specifically, authorisation is sought for chickens for fattening, chickens reared for laying and minor poultry species.

According to the Applicant, *6-phytase* is the active substance of APSA PHYTAFEED® *produced by Komagataella pastoris appa T75* (CGMCC 12056). The Applicant expresses the phytase enzymatic activity in units (U), where 'one U is the amount of enzyme which releases one micromole of inorganic phosphate from phytate per minute at pH 5.5 and 37°C'.

The product is intended to be marketed as a granulated and a liquid formulation having a guaranteed minimum *phytase* activity of 20,000 U/g (APSA PHYTAFEED® 20000 GR) and of 20,000 U/mL (APSA PHYTAFEED® 20000 L). APSA PHYTAFEED® is intended to be included into feedingstuffs directly and/or through premixtures to obtain a minimum activity of 250 U/kg feedingstuffs.

For the determination of *phytase* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant applied a modified protocol of the EN ISO 30024 standard method. Upon request of the EURL the Applicant applied (i) the ring-trial validated colorimetric EN ISO 30024 standard method for the quantification of the *phytase* activity in *feedingstuffs*, (ii) the ring-trial validated colorimetric method (VDLUFA 27.1.3) for the quantification of the *phytase* activity in *premixtures* and (iii) the ring-trial validated colorimetric method (VDLUFA 27.1.4) for the quantification of the *phytase* activity in the *feed additives*. Comparable results and method performance characteristics were obtained and demonstrate the applicability of these methods to the determination of phytase activity in *feed additive*, *premixtures* and *feedingstuffs* of the product under investigation.

Based on the performance characteristics provided the EURL recommends for official control the colorimetric methods mentioned above for the quantification of *phytase* activity in the *feed additive*, *premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.