

ADOPTED: 7 May 2020

doi: 10.2903/j.efsa.2020.6141

## Safety and efficacy of OptiPhos<sup>®</sup> PLUS for poultry species for fattening, minor poultry species reared for breeding and ornamental birds

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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 OptiPhos PLUS<sup>®</sup> to be used as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening, minor poultry species reared for laying, minor poultry species reared for breeding and ornamental birds. The active substance is 6-phytase produced by a genetically modified strain of *Komagataella phaffii*. The FEEDAP Panel concludes that the genetic modification of the production strain does not give rise to safety concerns. Based on the tolerance studies provided, the Panel concludes that the additive is safe for the target species under the conditions of use with a wide margin of safety. The additive is not a skin irritant. It is found to be a dermal sensitiser. The FEEDAP Panel cannot conclude on the eye irritation potential of the additive. Owing to the proteinaceous nature of the active substance, the additive is considered a respiratory sensitiser. The FEEDAP Panel concludes that the additive has the potential to be efficacious in increasing the phosphorus utilisation in chickens for fattening at the level of 250 FTU/kg feed. These conclusions can be extended to turkeys for fattening and chickens and turkeys reared for laying/breeding and extrapolated to all minor poultry species and other avian species up to the point of lay.

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**Keywords:** OptiPhos PLUS<sup>®</sup>, chickens for fattening, *Komagataella phaffii*, Safety, Efficacy, QPS, extrapolation

**Requestor:** European Commission

**Question number:** EFSA-Q-2019-00303

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**Suggested citation:** EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kos 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, Cocconcelli PS, Glandorf B, Herman L, Prieto MM, Saarela M, Anguita M, Brozzi R, Cazorla YG, Galobart J, Holczknecht O, Manini P, Pettenati E, Tarrés-Call J and Pizzo F, 2020. Scientific Opinion on the safety and efficacy of OptiPhos® PLUS for poultry species for fattening, minor poultry species reared for breeding and ornamental birds. *EFSA Journal* 2020;18(6):6141, 15 pp. <https://doi.org/10.2903/j.efsa.2020.6141>

**ISSN:** 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, an agency of 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 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 Huvepharma EOOD<sup>2</sup> for authorisation of the product OptiPhos® PLUS (6-phytase), when used as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening, minor poultry species reared for laying, minor poultry species reared for breeding and ornamental birds (category: zootechnical additives; functional group: digestibility enhancer).

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 17 July 2019.

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 OptiPhos® PLUS (6-phytase), when used under the proposed conditions of use (see Section 3.1.3.3).

### 1.2. Additional information

OptiPhos® PLUS (6-phytase) produced by a genetically modified strain of *Komagataella phaffii* (DSM 32854) has not been previously authorised as a feed additive 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<sup>3</sup> in support of the authorisation request for the use of OptiPhos® PLUS (6-phytase) as a feed additive.

The FEEDAP Panel used the data provided by the applicant to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the OptiPhos® PLUS (6-phytase) in animal feed. The Executive Summary of the EURL report can be found in Annex A.<sup>4</sup>

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of OptiPhos® PLUS (6-phytase) is in line with the principles laid down in Regulation (EC) No 429/2008<sup>5</sup> and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012a,b,c), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the

<sup>1</sup> 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.

<sup>2</sup> Huvepharma EOOD, 3A Nikolay Haytov Str. Sofia (Bulgaria).

<sup>3</sup> FEED dossier reference: FAD-2019-0023.

<sup>4</sup> The full report is available on the EURL website: [https://ec.europa.eu/jrc/sites/jrcsh/files/finrep\\_fad-2019-0023\\_optiphos\\_PLUS\\_avian.docx\\_.pdf](https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2019-0023_optiphos_PLUS_avian.docx_.pdf)

<sup>5</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.

assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

### 3. Assessment

The additive OptiPhos® PLUS is a preparation of 6-phytase (Enzyme Commission Number 3.1.3.26) produced by a genetically modified strain of *Komagataella phaffii* (DSM 32854) (previously *Pichia*). It is proposed to be used as a zootechnical additive (functional group: digestibility enhancers) for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening, minor poultry species reared for laying, minor poultry species reared for breeding and ornamental birds.

#### 3.1. Characterisation

##### 3.1.1. Characterisation of the production organism

The additive is produced by the genetically modified strain of *K. phaffii* which is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) with deposition number DSM 32854.<sup>6</sup>

The taxonomical identification of the production strain as *K. phaffii* was achieved [REDACTED]

##### 3.1.2. Characteristics of the recipient microorganism

[REDACTED]

##### 3.1.3. Characteristics of the inserted sequences

[REDACTED]

##### 3.1.4. Description of the genetic modification process

[REDACTED]

##### 3.1.5. Manufacturing process

The active substance of OptiPhos® PLUS is produced by fermentation with *Komagataella phaffii* DSM 32854. [REDACTED]

##### 3.1.6. Characterisation of the additive

The additive is available in three formulations:

<sup>6</sup> Technical dossier/Section II/Annex\_II\_27.

- OptiPhos® PLUS 5000 G (granular)
- OptiPhos® PLUS 5000 CT (coated)
- OptiPhos® PLUS 5000 L (liquid)

All the three formulations contain a minimum of 5,000 FTU<sup>7</sup> /g.

OptiPhos® PLUS 5000 G is a light beige to brown granular form that contains 6-phytase (3.25% w/w), pregelatinised starch (1.5% w/w) and wheat meal (up to 100% w/w).

OptiPhos® PLUS 5000 CT is a light beige to brown particles form that contains 6-phytase (4.3% v/w), distilled monoglyceride (10% w/w), palm oil (10% w/w), wheat meal (40.7% w/w) and rice hulls (up to 100% w/w).

OptiPhos® PLUS 5000 L (density 1.10–1.20 g/mL) is a light yellow to greenish liquid form that contains 6-phytase (4.33% v/v), sodium benzoate (0.25% w/v), hydrochloric acid (0.02% v/v), glycerin (45% v/v) and purified water (up to 100% v).

The applicant provided results on batch-to-batch variation analysis performed on six batches of each formulation. The 6-phytase activity for the G formulation ranged from 5,910 to 6,690 FTU/g, for the CT formulation from 5,790 to 6,650 FTU/g and for the L formulation 6,640 to 6,940 FTU/g. The results demonstrated that the product is manufactured in compliance with the proposed specifications.<sup>8,9,10</sup>

The presence of impurities (arsenic (< 2 mg/kg), lead (< 10 mg/kg), cadmium (< 1 mg/kg), mercury (< 0.1 mg/kg), aflatoxins total (< 1.5 µg/kg) dioxins and polychlorinated biphenyl (PCB)) was evaluated in three batches for each formulation of the product and the results were in compliance with the proposed specifications for the additive.<sup>11</sup>

Microbial contamination was analysed on the six batches for each formulation (*Salmonella*, absent in 25 g).<sup>12</sup> Analysis on additional three batches for each formulation of the additive were provided regarding the possible presence of microbial impurities (total aerobic microbial count ranging from  $1 \times 10^2$  to  $5 \times 10^2$  CFU/g, total coliforms < 30 CFU/g, *E. coli* and *Salmonella* absent in 25 g).<sup>13</sup> The results followed the specifications set by JECFA for enzymes preparations used in food processing.

The applicant provided analysis on the content of impurities (arsenic < 0.05 mg/kg, lead, cadmium < 0.1 mg/kg and lead < 1 mg/kg) on three batches of the active substance (6-phytase liquid concentrate). Microbial contamination was also analysed on the same batches (*Salmonella* absent in 25 mL).<sup>14</sup>

Based on the results, no concern arises on possible presence of impurities in the final product.

The absence of viable cells of the producing strain in the OptiPhos® PLUS was evaluated in a study performed on three batches analysed in triplicate. [REDACTED]

The absence of recombinant DNA in the feed additive was demonstrated [REDACTED]

The particle size was measured by sieve analysis in three batches of OptiPhos® PLUS 5000 G and CT.<sup>17,18</sup> The results indicated that 95.6% of the particles was between 800 and 100 µm and 4% was below 100 µm in the granular formulation and, 96.4% of the particles was between 1,000 and 250 µm and 3.4% was below 250 µm in the coated formulation.

<sup>7</sup> One FTU unit as described in the EN ISO 30024 is defined as 'the amount of enzyme that releases 1 µmol of inorganic phosphate from sodium phytate per minute under reaction conditions of pH 5.5 and 37°C'.

<sup>8</sup> Technical dossier/Section II/Annex\_II\_13.

<sup>9</sup> Technical dossier/Section II/Annex\_II\_14.

<sup>10</sup> Technical dossier/Section II/Annex\_II\_15.

<sup>11</sup> Technical dossier/Section II/Annex\_II\_20.

<sup>12</sup> Technical dossier/Section II/Annex\_II\_21.

<sup>13</sup> Technical dossier/Section II/Supplementary information\_RTQ\_3.

<sup>14</sup> Technical dossier/Section II/Annex\_II\_16.

<sup>17</sup> Technical dossier/Section II/Annex\_II\_22.

<sup>18</sup> Technical dossier/Section II/Annex\_II\_24.

The dusting potential (Stauber–Heubach method) measured in six batches of OptiPhos® PLUS 5000 G and in three batches of OptiPhos® PLUS 5000 CT ranged from 0 to 5 mg/m<sup>3</sup> and from 190 to 220 mg/m<sup>3</sup>, respectively.<sup>19,20,21</sup>

Bulk and tapped density, measured in three batches of OptiPhos® PLUS 5000 G was 550 and 630 kg/m<sup>3</sup> and of OptiPhos® PLUS 5000 CT was 520 and 600 kg/m<sup>3</sup>.<sup>17,18</sup>

Density of OptiPhos® PLUS 5000 L (liquid formulation) was 0.001 kg/cm<sup>3</sup> (average of six batches); viscosity was 12.5 cP (average of three batches) and pH was 4.6 (average of six batches).<sup>10,22,23</sup>

### 3.1.7. Stability and homogeneity

#### 3.1.7.1. Shelf-life of the additive

Three batches for each formulation of the additive were tested.

OptiPhos® PLUS 5000 G was stored in a small size container, consisting of primary polyethylene bag and secondary paper bag, at two different conditions: 25°C (relative humidity – RH 60%) for 24 months and at 40°C (RH 75%) for 6 months. The loss in 6-phytase content ranged between 6.9% and 11.1% and between 9.8% and 14.2% after 24 and 6 months storage, respectively.<sup>24</sup>

OptiPhos® PLUS 5000 CT was stored at the same conditions as above. The loss in 6-phytase content ranged between 7.1% and 10.4% and between 8.9% and 13.6% after 24 and 6 months storage, respectively.<sup>25</sup>

OptiPhos® PLUS 5000 L was stored in a small size plastic bottle, containing 200 ml of the product, at two different conditions: 25°C for 12 months and at 40°C for 6 months. The loss in 6-phytase content ranged between 8.8% and 11.4% and between 19.6% and 25.4% after 12 and 6 months storage, respectively.<sup>26</sup>

#### 3.1.7.2. Stability in vitamin/mineral premixtures

Three batches of OptiPhos® PLUS 5000 G and CT were tested when stored in a commercial ready-to-use vitamin/mineral premixture for poultry containing choline chloride (250,000 mg/kg).<sup>13</sup> The 6-phytase inclusion level was 100 FTU/g premixture. After storage in polyethylene bags at 25°C for 6 months, the loss in 6-phytase content ranged between 9.3% and 17.4% and between 2.7% and 12.7% for OptiPhos® PLUS G and CT, respectively. After storage at 40°C for 6 months, the loss in 6-phytase content ranged between 7.1% and 24.4% and between 9% and 18.6% for OptiPhos® PLUS G and CT, respectively.

#### 3.1.7.3. Stability in feedingstuffs

Three batches for each formulations of the additive were tested for conditioning/pelleting and storage stability in feed for chickens for fattening when included at 250 FTU/kg feed. The basal diet consisted of maize, soybean groats, wheat and sunflower expeller.

##### *OptiPhos® PLUS 5000 G*

After 3 months packed in polyethylene bags at 25°C, the decrease of 6-phytase level in the mash test diets was between 10.5% and 19.2%, and after 3 weeks at 40°C between 19.9% and 26.6%.<sup>27</sup>

In pelleted test diets, the decrease of 6-phytase level after a period of 3 months of storage in polyethylene bags at 25°C was between 2.6% and 10.8%, and for a period of 3 weeks at 40°C between 14.3% and 17.3%.

From the conditioning with saturated steam (85°C) recoveries for the 6-phytase, contents in the pellets vs the mash feeds were between 80.2% and 86.6%.

##### *OptiPhos® PLUS 5000 CT*

After 3 months packed in polyethylene bags at 25°C, the decrease of 6-phytase level in the mash test diets was between 11.5% and 14.3%, and after 3 weeks at 40°C between 18.5% and 21.3%.<sup>28</sup>

<sup>19</sup> Technical dossier/Section II/Annex\_II\_23.

<sup>20</sup> Technical dossier/Section II/Supplementary information\_RTQ\_1.

<sup>21</sup> Technical dossier/Section II/Annex\_II\_25.

<sup>22</sup> Technical dossier/Section II/Annex\_II\_26.

<sup>23</sup> Technical dossier/Section II/Supplementary information\_RTQ\_2.

<sup>24</sup> Technical dossier/Section II/Annex\_II\_56.

<sup>25</sup> Technical dossier/Section II/Annex\_II\_57.

<sup>26</sup> Technical dossier/Section II/Annex\_II\_58.

<sup>27</sup> Technical dossier/Section II/Annex II.56.

<sup>28</sup> Technical dossier/Section II/Annex II.57.

In pelleted feed when packed in polyethylene bags, the decrease of 6-phytase level after a period of 3 months of storage at 25°C was between 9.8% and 11.2%, and for a period of 3 weeks at 40°C between 16.0% and 17.8%.

From the conditioning with saturated steam (90°C), recoveries for the 6-phytase, contents in the pelleted feeds versus the mash feeds were between 80.5% and 88.5%.

#### *OptiPhos® PLUS 5000 L*

In pelleted feed when packed in polyethylene bags, the decrease of 6-phytase level after a period of 3 months of storage at 25°C was not more than 18.7%, and for a period of 3 weeks at 40°C – not more than 31.8%.<sup>29</sup>

#### **3.1.7.4. Homogeneity**


The capacity of OptiPhos® PLUS 5000 G, CT and L to homogeneously distribute in feedingstuffs was studied by analyzing 10 subsamples of the feeds prepared for stability testing (three batches per formulation). Samples analysed showed an average coefficient of variation (CV) of 10.5% (range 8–13.8%), 8.1% (range 7.4–9.1%) and 15.4% (range 9.2–21.8%) for OptiPhos® PLUS 5000 G, CT and L, respectively.<sup>30</sup>

#### **3.1.7.5. Conditions of use**

The product is intended to be used in feed for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening, minor poultry species reared for laying, minor poultry species reared for breeding and ornamental birds at a minimum inclusion level of 250 FTU/kg complete feed. The recommended inclusion level ranges from 250 to 500 FTU/kg complete 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 qualified presumption of safety (QPS) approach to safety assessment when used for enzyme production (EFSA BIOHAZ Panel, 2007, 2020). 

The production strain was identified as *K. phaffii* and the product OptiPhos® PLUS is free from viable cells of the production organism and its recombinant DNA.

Therefore, the additive does not pose any safety concern regarding the genetic modification of the production strain.

#### **3.2.2. Toxicological studies**

The FEEDAP Panel notes that all the toxicological studies were performed not with the final formulations of the additive but with a concentrate form of 6-phytase (OptiPhos® PLUS Concentrate – 300,000 FTU/g). However, considering the nature of the other components of the final formulations of the additive, the FEEDAP Panel considers that the conclusions reached for the concentrate apply to the final formulations of the additive.

##### **3.2.2.1. Genotoxicity**

###### *Bacterial reverse mutation assay*

OptiPhos® PLUS Concentrate (300,000 FTU/g) was tested for the induction of reverse mutations in *Salmonella* Typhimurium tester strains TA98, TA100, TA1535 and TA1537 and in *E. coli* strain WP2uvrA.<sup>31</sup> The experimental protocol was in line with Organisation for Economic Co-operation and Development (OECD) guideline 471 (1997) and in compliance with Good Laboratory Practice (GLP). No increase in revertant colony numbers was observed at any dose and with any tester strains. The Panel concluded that the test item did not induce gene mutations in bacteria under the experimental conditions employed in this study.

<sup>29</sup> Technical dossier/Section II/Annex II.58.

<sup>30</sup> Technical dossier/Section II/Annexes II.56-58.

<sup>31</sup> Technical dossier/Section III/Annex\_III\_4.



### *In vivo mammalian erythrocyte micronucleus test*

OptiPhos® PLUS Concentrate (300,000 FTU/g) was tested for the induction of micronuclei in an *in vivo* micronucleus test in bone marrow erythrocytes of NMRI mice, performed in GLP and compliance with OECD guideline 474 (2016).<sup>32</sup> No clinical signs of systemic toxicity were observed in treated animals. No significant cytotoxicity was induced in the bone marrow, since comparable frequencies of polychromatic erythrocytes were reported between negative controls and treated animals. No significant increase in the frequency of micronuclei was detected in treated animals, while mice dosed with cyclophosphamide responded with statistically significant increase of micronuclei. The Panel concluded that OptiPhos® PLUS did not induce micronuclei in bone marrow erythrocytes when tested up to the maximum recommended dose level.

#### **3.2.2.2. Subchronic toxicity study**

Wistar rats (10/sex and group, 15/sex and control and highest dose group) received the test item by oral gavage as a water suspension (0.5 mL/100 g body weight (bw) per day) at dose levels of 0 (control), 100, 500 or 1,000 mg OptiPhos® PLUS Concentrate/kg bw per day for 90 days.<sup>33</sup> The study was conducted in GLP and compliance with OECD guideline 408.

No treatment-related clinical signs were observed. All animal survived the treatment period.

No effects were observed on behaviour, body weight, feed intake, haematology, clinical parameters, urine analysis, gross pathology and histopathological examination. From this study a no observed adverse effect level (NOAEL) of 1,000 mg/kg bw per day, the highest dose tested, was identified.

#### **3.2.3. Safety for the target species**

##### **3.2.3.1. Safety for chickens for fattening<sup>34</sup>**

A total of 1,170 one-day-old male chickens for fattening (Ross 308) were distributed in 78 pens (containing 15 animals/pen) and randomly allocated to three dietary treatments (26 pens/replicates per treatment). Three basal diets (starter, grower and finisher) based on maize and soybean meal were either not supplemented (control) or supplemented with OptiPhos® PLUS to provide 500 (1× maximum recommended dose) or 50,000 (100×) FTU per kg feed (confirmed by analysis). The diets were offered in pelleted form for 35 days. Mortality and health status were checked every day. Animals were weighed on days 0, 14, 28 and 35 (pen basis), feed intake was registered per pen and average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (FCR) calculated. ADG and ADFI of the control group were compared with the ones of the supplemented treatment groups in a non-inferiority test (t-test with a margin added, 80% power) using predefined non-inferiority margins of 3 g/day for ADG and 5 g/day for ADFI.<sup>35</sup> In addition, an analysis of variance (ANOVA) was done with the performance data (pen basis) considering the treatment as fixed effect and initial body weight as covariate.<sup>36</sup> The pen was the experimental unit. When ANOVA results were significant, group means were compared by Tukey's and Dunnett's tests. The significance level was set at  $p \leq 0.05$ .

Mortality including culling was 2.1%, not treatment-related (number of death/culled birds were 11, 8 and 6 for control, 1x and 100x, respectively) and not analysed statistically. ADG and ADFI of 1x and 100x treatment groups were not statistically inferior when compared to the control group considering the non-inferiority margins established. There were statistically significant differences in body weight at the end of the study being the body weight of 100× group higher compared with the other two groups (2,676 g vs. 2,609 g and 2,596 g in control and 1× group, respectively). ADG was significantly improved in the 100x group compared to the other two groups (75.1 g/day vs. 73.3 g/day and 73.0 g/day in control and 1x group, respectively). FCR corrected for mortality was significantly improved in 1× and 100× groups compared to the control group (1.42 and 1.40 vs. 1.43, respectively).

##### **3.2.3.2. Conclusions on safety for the target species**

The FEEDAP Panel concludes that OptiPhos® PLUS is safe for chickens for fattening at the recommended level of 500 FTU/kg complete feed with a margin of safety of at least 100-fold. This conclusion is extended to chickens reared for laying and extrapolated to turkeys for fattening, turkeys

<sup>32</sup> Technical dossier/Section III/Annex\_III\_5.

<sup>33</sup> Technical dossier/Section III/Annex\_III\_9.

<sup>34</sup> Technical dossier/Section II/Annex\_III\_1.

<sup>35</sup> Technical dossier/Supplementary information January 2020/Annex RTQ\_5.2.

<sup>36</sup> Technical dossier/Supplementary information January 2020/Annex RTQ\_5.1.

reared for breeding, minor poultry species for fattening, minor poultry species reared for laying, minor poultry species reared for breeding and ornamental birds.

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

### 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 for the consumers. 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.

The FEEDAP Panel concludes that the use of OptiPhos PLUS® in animal nutrition under the proposed conditions of use is safe for the consumers.

### 3.2.5. Safety for user

#### 3.2.5.1. Effects on the respiratory system

No specific studies were provided by the applicant regarding the effects of the additive on the respiratory system. Although the dusting potential of the solid forms are low/negligible, considering the proteinaceous nature of the active substance, the additive in all forms, is considered as respiratory sensitiser.

#### 3.2.5.2. Effects on the skin and eyes

No data were provided on the final formulations.

The skin irritation potential of OptiPhos® PLUS Concentrate was tested in a GLP study performed according to OECD guideline 439, which showed that it is not a skin irritant.<sup>37</sup>

The eye irritation potential of OptiPhos® PLUS Concentrate was tested in a GLP study performed according to OECD guideline 438.<sup>38</sup> OptiPhos® PLUS Concentrate has been categorised as 'no prediction can be made' and the FEEDAP Panel cannot conclude on eye irritation potential.

In a GLP skin sensitisation study following local lymph node assay (LLNA) following OECD guideline 429, OptiPhos® PLUS Concentrate was found to be a skin sensitiser.<sup>39</sup>

#### 3.2.5.3. Conclusions on safety for the user

OptiPhos® PLUS Concentrate is not a skin irritant, but it is a dermal sensitiser. The components used in the final formulation are not expected to modify the toxicological profile of the test item used to perform the studies. Owing to the proteinaceous nature of the active substance, the additive is considered a respiratory sensitiser. The FEEDAP Panel cannot conclude on eye irritation of the additive.

### 3.2.6. Safety for the environment

The production strain and its DNA were not detected in the final product. The additive does not raise safety concerns for the environment regarding 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

### 3.3.1. Efficacy for chickens for fattening

Three balance trials, which included performance, done in chickens for fattening were submitted for the assessment. The three trials shared a similar experimental design.

<sup>37</sup> Technical dossier/Section III/Annex\_7.

<sup>38</sup> Technical dossier/Section III/Annex\_8.

<sup>39</sup> Technical dossier/Section III/Annex\_6.

In the first trial<sup>40,41</sup> 600 one-day-old male chickens for fattening (Ross 308) were distributed in 40 pens in groups of 15 animals, in the second<sup>42,43</sup> 528 one-day-old male chickens for fattening (Ross 308) were distributed in 48 pens in groups of 11 animals and in the third<sup>44,45</sup> 480 one-day-old male chickens for fattening (Ross 308) were distributed in 24 pens in groups of 20 animals. Basal diets based on corn, wheat, and soybean meal were either not supplemented (control) or supplemented with OptiPhos PLUS® to provide 250 or 500 FTU per kg feed (confirmed by analysis). In all three trials, a positive control diet with higher content of phosphorus (P) and calcium (Ca) was also considered. In the first trial, P and Ca content (mg/kg feed) was 7.8/6.1 and 7.6/6.2 (analysed values) in the grower and finisher phases, respectively. In the second trial, P and Ca content was 8.3/6.4 and 7.1/6.1 in the grower and finisher phases, respectively. In the third trial, the P and Ca content was 8.1/6.5 and 7.5/6.4 in the grower and finisher phases, respectively. Diets were offered in pelleted form for 28 (trial 1) or 30 days (trials 2 and 3) and contained titanium dioxide (in trials 1 and 2) or silicon dioxide (in trial 3) as external markers. Mortality and health status were checked every day and dead animals were necropsied. Animals were weighed on pen basis on days 7, 21 and 35 in trial 1, on days 1, 5, 21 and 35 in trial 2 and on days 0, 5, 21 and 35 in trial 3. Feed intake was measured throughout the study period and feed to gain ratio was calculated. In trial 1, the balance study was conducted on days 29–32 of life, in trial 2 on day 31–33 of life and in trial 3 on days 30–36 of life. In all the three studies, on day 21 of age two birds per pen were killed to collect tibia samples to study bone ash and phosphorus content (in trial 2 only for ash content). Feed and excreta samples were analysed for total phosphorus and/or phytate phosphorus to determine the utilisation/digestibility. An ANOVA was done with the data and group means were compared with the Tukey's test. The significance level was set at  $p \leq 0.05$ .

Results of the performance and digestibility of the three trials are reported in Table 1.

**Table 1:** Effects of OptiPhos® PLUS on the performance and the utilisation of total P/digestibility phytate P in chickens for fattening

Tria	Groups (FTU/kg feed)	Average daily feed intake (g)	Final body weight (g)	Feed to gain ratio	Mortality and culling (n.)	Tibia content in % DM		Utilisation of P (%)	Digestibility of Phytate P (%)
						Ash	P		
1	Negative control	92 <sup>b</sup>	1,830 <sup>b</sup>	1.78 <sup>b</sup>	7	37.39	6.14 <sup>c</sup>	43.31 <sup>b</sup>	65.76 <sup>b</sup>
	250	103 <sup>a</sup>	2,063 <sup>a</sup>	1.68 <sup>b</sup>	1	39.63	6.90 <sup>b</sup>	49.61 <sup>a</sup>	80.35 <sup>a</sup>
	500	106 <sup>a</sup>	2,192 <sup>a</sup>	1.61 <sup>b</sup>	2	41.47	7.32 <sup>ab</sup>	52.82 <sup>a</sup>	84.58 <sup>a</sup>
	Positive control	108 <sup>a</sup>	2,185 <sup>a</sup>	1.65 <sup>a</sup>	4	41.61	7.78 <sup>a</sup>	36.59 <sup>c</sup>	47.76 <sup>c</sup>
2	Negative control	114 <sup>b</sup>	2,121 <sup>b</sup>	1.58 <sup>c</sup>	4	40.9 <sup>c</sup>		47.1 <sup>b</sup>	
	250	120 <sup>a</sup>	2,346 <sup>a</sup>	1.51 <sup>b</sup>	1	45.0 <sup>b</sup>		53.1 <sup>a</sup>	
	500	122 <sup>a</sup>	2,385 <sup>a</sup>	1.51 <sup>b</sup>	3	47.1 <sup>a</sup>		51.5 <sup>ab</sup>	
	Positive control	119 <sup>a</sup>	2,350 <sup>a</sup>	1.48 <sup>a</sup>	4	47.4 <sup>a</sup>		48.9 <sup>ab</sup>	
3	Negative control	90.2 <sup>c</sup>	2,004 <sup>c</sup>	1.50 <sup>a</sup>	6	41.6 <sup>c</sup>	17.3 <sup>c</sup>	49.9 <sup>a</sup>	69.7 <sup>c</sup>
	250	102.6 <sup>b</sup>	2,293 <sup>b</sup>	1.48 <sup>ab</sup>	8	45.5 <sup>b</sup>	17.5 <sup>bc</sup>	52.8 <sup>a</sup>	85.9 <sup>b</sup>
	500	104.9 <sup>ab</sup>	2,323 <sup>ab</sup>	1.47 <sup>ab</sup>	4	46.5 <sup>b</sup>	17.7 <sup>ab</sup>	51.8 <sup>a</sup>	95 <sup>a</sup>
	Positive control	108.8 <sup>a</sup>	2,425 <sup>a</sup>	1.46 <sup>b</sup>	3	49.4 <sup>a</sup>	17.9 <sup>a</sup>	38.7 <sup>b</sup>	56.6 <sup>d</sup>

a,b,c: Within a trial mean values within a column with a different superscript are significantly different  $p \leq 0.05$ .

<sup>40</sup> Technical dossier/Section IV/Annex\_1.

<sup>41</sup> Technical dossier/Section IV/Supplementary information\_RTQ\_8.1.

<sup>42</sup> Technical dossier/Section IV/Annex IV\_2.

<sup>43</sup> Technical dossier/Section IV/Supplementary information\_RTQ\_8.2.

<sup>44</sup> Technical dossier/Section IV/Annex IV\_3.

<sup>45</sup> Technical dossier/Section IV/Supplementary information\_RTQ\_8.3.

In the three trials, the birds fed with the phytase showed increases in total P utilisation and phytate P digestibility from the level of 250 FTU/kg feed. In the three trials significant effects on the bone mineralisation (ash/P content) were found from the level of 250 FTU/kg feed.

Although the performance was improved by the addition of the additive at 250 FTU/kg feed, the FEEDAP Panel considered these data as supporting evidence since these trials lasted less time than normally requested to evaluate animal performance.

### 3.3.1.1. Conclusions on efficacy

The FEEDAP Panel concludes that additive has the potential to be efficacious in increasing the P utilisation in chickens for fattening at the level of 250 FTU/kg feed.

The conclusions on the chickens for fattening can be extended to chickens reared for laying at the corresponding dose.

The mode of action of the phytase is well known and can be considered to be similar in all poultry/avian species. Therefore, the conclusions drawn in chickens for fattening can be extrapolated to turkeys for fattening and turkeys reared for laying/breeding and to all minor poultry species up to the point of lay and to ornamental birds.

## 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<sup>46</sup> 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.

All the three formulations of OptiPhos® PLUS (G, CT and L) are considered equivalent in terms of safety and efficacy.

The additive is safe for chickens for fattening or reared for laying/breeding and for minor poultry species for fattening or reared for laying/breeding and in ornamental birds at the recommended level of 500 FTU/kg feed.

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

The additive in its different forms is not irritant for skin but is a dermal and respiratory sensitiser. The FEEDAP Panel cannot conclude on eye irritation of the additive.

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

OptiPhos® PLUS has the potential to improve the utilisation of phosphorus in the diets in chickens for fattening or reared for laying, in turkeys for fattening or reared breeding and for minor poultry species for fattening or reared for laying/breeding and in ornamental birds at 250 FTU/kg feed.

## 5. Documentation as provided to EFSA/Chronology

Date	Event
03/04/2019	Dossier received by EFSA. OptiPhos PLUS® (6-Phytase). Submitted by Huvepharma EOOD
30/04/2019	Reception mandate from the European Commission
17/07/2019	Application validated by EFSA – Start of the scientific assessment
20/09/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation, safety and efficacy</i>
22/10/2019	Comments received from Member States
19/11/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
31/01/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
07/05/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

<sup>46</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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## Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ANOVA	analysis of variance
bw	body weight

CV	coefficient of variation
DM	dry matter
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EURL	European Union Reference Laboratory
FCR	feed conversion ratio
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practice
LLNA	local lymph-node assay
LOD	limit of detection
LOQ	limit of quantification
MW	molecular weight
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
RH	relative humidity

## **Annex A – Executive summary of the evaluation report of the European Union Reference Laboratory on the analytical methods submitted for the preparation of 6-phytase (OptiPhos PLUS®)**

In the current application, authorisation of a Preparation of 6-phytase (EC 3.1.3.26) is sought under Article 4(1) for all porcine species under the category/functional group 4(a) “zootechnical additives”/ “digestibility enhancers”.

According to the Applicant, the active agent is 6-phytase. The phytase activity is expressed in phytase units (FTU). One FTU unit as described in the EN ISO 30024 is defined as “the amount of enzyme that releases 1 µmol of inorganic phosphate from sodium phytate per minute under reaction conditions of pH 5.5 and 37 °C”.

The product is intended to be marketed in solid and liquid formulations denoted as OptiPhos® PLUS 5000 G, 5000 CT and 5000 L with a guaranteed minimum 6-phytase activity of 5000 FTU/g. The product is intended to be included through premixtures or directly in feedingstuffs to obtain a minimum activity of 250 FTU/kg feedingstuffs.

For the quantification of the phytase activity the Applicant submitted the ring-trial validated colourimetric standard methods EN ISO 30024 (for feedingstuffs) and VDLUFA 27.1.4 (for the feed additive). In addition, the Applicant applied also the VDLUFA 27.1.4 with minor experimental modifications to analyse the premixtures and obtained similar method performance characteristics. However the EURL is aware of the ring-trial validated VDLUFA 27.1.3 method specifically describing the preparation of premixtures for quantification of the phytase activity according to EN ISO 30024.

Based on the performance characteristics available the EURL recommends for official control the colourimetric methods mentioned above for the quantification of the 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.