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Safety and efficacy of OptiPhos[®] PLUS for suckling and weaned piglets, pigs for fattening, sows, other minor pig species for fattening and other minor reproductive pig species

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Abstract

Following a request from the European Commission, the EFSA Panel on Additives and products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of OptiPhos[®] PLUS (6-phytase) as a zootechnical feed additive for suckling and weaned piglets, pigs for fattening, sows, other minor pig species for fattening and other minor reproductive pig species. The additive is a preparation of 6-phytase produced by a genetically modified strain of *Komagataella phaffii* and has been previously assessed by the FEEDAP Panel in the context of two applications for its use in different species/categories. The Panel concluded in those opinions that the production strain is safe, and that the use of the additive as a feed additive would raise no safety concerns for the consumers and the environment. The additive was also considered not to be skin irritant but was found to be a dermal sensitiser. The FEEDAP Panel could not 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. Based on the tolerance studies provided, the Panel concluded that the additive is safe for the target species under the conditions of use with a wide margin of safety and therefore the conclusion were extended to suckling piglets and extrapolated to other minor pig species for fattening and to other minor reproductive pig species. The FEEDAP Panel concluded that the additive has the potential to be efficacious in weaned piglets, pigs for fattening and in sows at the level of 250 FTU/kg feed and this conclusion were extended to suckling piglets and extrapolated to other minor pig species for fattening and to other minor reproductive pig species.

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1. Introduction

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

Regulation (EC) No 1831/2003¹ 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 EOOD² for authorisation of the product OptiPhos® PLUS (6-phytase), when used as a feed additive for suckling and weaned piglets, pigs for fattening, sows, other minor pig species for fattening, other minor reproductive pig 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). The particulars and documents in support of the application were considered valid by EFSA as of 28 August 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).

1.2. Additional information

OptiPhos® PLUS (6-phytase) produced by a genetically modified strain of *Komagataella* (previously *Pichia*) *phaffii* (DSM 32854) has not been previously authorised as a feed additive in the European Union.

EFSA issued two opinions on OptiPhos® PLUS (6-phytase) produced by a genetically modified strain of *K. phaffii* (DSM 32854). One for the use as a feed additive in poultry species for fattening, chickens reared for laying, minor poultry species reared for breeding and ornamental birds (EFSA FEEDAP Panel, 2020a) and another one for the use in laying hens, turkeys for breeding, chickens for breeding, minor poultry species for egg production purposes and breeding (EFSA FEEDAP Panel, 2020b).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier³ 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 6-phytase 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 OptiPhos® PLUS (6-phytase) is in line with the principles laid down in Regulation (EC) No 429/2008⁵ 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 identity, characterisation and conditions of

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 Huvepharma EOOD, 3A Nikolay Haytov Str. Sofia (Bulgaria).

³ FEED dossier reference: FAD-2019-0042.

⁴ The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2019-0042_optiphos_PLUS_porcine.pdf

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.

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 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 (EC number 3.1.3.26; phytase) produced by a genetically modified strain of *K. phaffii* (DSM 32854). It is proposed to be used as a zootechnical additive (functional group: digestibility enhancer) for suckling and weaned piglets, pigs for fattening, sows, other minor pig species for fattening and other minor reproductive pig species.

3.1. Characterisation

The additive is produced by a genetically modified strain of *K. phaffii* (previously *Pichia phaffii*) deposited at the DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH with deposition number DSM 32854. It is available in three formulations, OptiPhos® PLUS 5000 G (granular), OptiPhos® PLUS 5000 CT (coated) and OptiPhos® PLUS 5000 L (liquid). All the three formulations contain a minimum of 5,000 FTU/g. The additive was fully characterised, including the genetic modification of the production strain, in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2020).

3.1.1. Stability and homogeneity

The applicant provided data on the stability of the additive in a premixture and feed for pigs and on the homogeneity in feed which were not provided for previous EFSA evaluations (EFSA FEEDAP Panel, 2020).

3.1.1.1. Stability in vitamin–mineral premixtures

The stability of three batches of the granular and coated formulations of the additive was tested in a vitamin/mineral premixture for pigs (containing choline chloride).⁶ The phytase inclusion level was 100 FTU/g premixture. After storage in a polyethylene bags and in a secondary paper bag at 25°C for 6 months, the highest loss of activity was 16.7% and after 3 weeks at 40°C was 25.9% for OptiPhos® PLUS 5000 G; the corresponding values for OptiPhos® PLUS 5000 CT were 14.5% and 23.46.

3.1.1.2. Stability in feed

Three batches for each formulation of the additive were tested for conditioning/pelleting and storage stability in feed for pigs at an inclusion level of 250 FTU/kg feed.

OptiPhos® PLUS 5000 G

After 3 months packed in a polyethylene bags and in a secondary paper at 25°C, the decrease of phytase activity in the mash test diets was up to 18.1%, and after 3 weeks at 40°C up to 24.1%.⁷

In pelleted test diets, the decrease of phytase activity after a period of 3 months of storage in polyethylene bags at 25°C was between 9.3% and 13.1%, and for a period of 3 weeks at 40°C between 15.8% and 18.5%.

The recoveries of phytase activity in the pelleted feed (85°C) were between 88.3% and 90.8% that of the original mash feed.

OptiPhos® PLUS 5000 CT

After 3 months packed in a polyethylene bags and in a secondary paper at 25°C, the decrease of phytase activity in the mash test diets was between 10.4% and 13.1%, and after 3 weeks at 40°C between 18.5% and 21.3%.⁸

⁶ Technical dossier/Section II/Annex II_55.

⁷ Technical dossier/Section II/Annex II.56.

⁸ Technical dossier/Section II/Annex II.57.

In pelleted test diets, the decrease of phytase activity after a period of 3 months of storage in polyethylene bags at 25°C was between 11.5% and 12.8%, and for a period of 3 weeks at 40°C between 15.5% and 17.1%.

The recoveries of phytase activity in the pelleted feed (90°C) were between 83.4% and 87.7% that of the original mash feed.

OptiPhos® PLUS 5000 L

In pelleted feed when stored in a small size plastic bottle, the decrease of phytase activity after a period of 3 months of storage at 25°C was not more than 17.9%, and for a period of 3 weeks at 40°C not more than 30.3%.⁹

3.1.1.3. Homogeneity

The capacity of the three batches (for each formulation, the same batches analysed for stability) of OptiPhos® PLUS 5000 G, CT and L to homogeneously distribute was studied in feed by analysing sets of 9 subsamples of the feed supplemented at 250 FTU/kg feed. The coefficients of variation were up to 10.8%, 6.5% and 14.6% for OptiPhos® PLUS 5000 G, OptiPhos® PLUS 5000 CT and OptiPhos® PLUS 5000 L, respectively.¹⁰

3.1.2. Conditions of use

The additive is intended to be used in feed for suckling and weaned piglets, pigs for fattening, sows, other minor pig species for fattening and other minor reproductive pig species at a minimum inclusion level of 250 FTU/kg of complete feedingstuffs. The recommended inclusion level ranges from 250 to 500 FTU/kg feedingstuffs.

3.2. Safety

The safety aspects regarding the use of this additive in feed including the safety of the genetic modification of the production strain, the safety for the consumers, for the user and for the environment have been previously assessed (EFSA FEEDAP Panel, 2020a). The Panel concluded that the additive does not pose any safety concern regarding the genetic modification of the production strain. The use of the product as a feed additive raises no concerns for consumer safety and for the environment. Regarding the safety for the user the Panel concluded that the additive is not a skin irritant but is a dermal sensitiser. The FEEDAP Panel could not 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 is not aware of any new information that would lead to reconsider the conclusions drawn previously and considers that the extension of use to the new species for which the application is made would not have an impact on the safety aspects already evaluated. However, the safety for the new target species/categories sought in the current application needs to be addressed.

3.2.1. Safety for the target species

3.2.1.1. Safety for piglets

A total of 252 weaned piglets (Duroc x White Landrace, 28-day-old, average initial body weight 7 kg) were distributed in 63 pens (sex-mixed) in groups of 4 animals (2 males and 2 females) and allocated to 3 dietary treatments (21 replicates per treatment).^{11,12} Two basal diets (pre-starter and starter) were either not supplemented (control) or supplemented with the phytase from OptiPhos® PLUS to provide 500 (1× highest recommended dose) or 50,000 (100×) FTU/kg (confirmed by analysis). The pre-starter diet was supplemented with calcium (Ca) at 8.4 g/kg and phosphorus (P) at 5.3 g/kg, and the starter diet was supplemented with Ca at 8 g/kg and P at 5.2 g/kg. Diets were offered in mash form for 42 days. Mortality and health status were checked every day. Animals were individually weighed on days 1, 14 and 42, feed intake was registered per pen and feed conversion rate (FCR) calculated. A non-inferiority test was used to analyse the performance parameters. Performance parameters were further

⁹ Technical dossier/Section II/Annex II.58.

¹⁰ Technical dossier/Section II/Annexes II.56–58.

¹¹ Technical dossier/Section III/Annex III_1.

¹² Technical dossier/Supplementary information/Annex_RTQ_III_1.1.

analysed statistically by and analysis of variance (ANOVA) including treatment as the fixed effect and start body weight as a covariate. The pen was the statistical unit. Group means were compared with Tukey's test.

All piglets remained healthy throughout the study, except for one piglet in the 1× group which was culled due to a broken leg. The non-inferiority test showed no inferiority of average daily gain (ADG) and average daily feed intake (ADFI) in the 1× and 100× treatment groups when compared with the control group. No significant differences between treatments were observed in the final body weight, ADFI and ADG (control group values final body weight was 23.9 kg, ADFI was 642 g, ADG was 400 g). Significant differences were observed for FCR which decreased at the highest dose tested (50,000 FTU/kg) compared to the control and 1× group (1.61 and 1.60 vs 1.57).

Feeding the piglets with OptiPhos® PLUS up to 100-fold the maximum recommended dose did not have any negative effects on the performance parameters of weaned piglets.

3.2.1.2. Safety for sows

A total of 72 sows (Topigs GY x Dutch Landrace, primiparous and multiparous) were distributed to 4 dietary treatments (18 replicates per treatment), based on parity, body weight and back fat thickness.^{13,14} The study was conducted with two groups of sows in two rounds during one full cycle of gestation and lactation, until detection of the subsequent oestrus. The study lasted approximately 21 weeks (i.e. 114 days in gestation, 26 days in lactation and 5 days of weaning to first oestrus interval). The four dietary treatments (gestation and lactation periods) were obtained by either not supplementing the gestation and lactation diets (control, low P content) or supplementing them with the phytase from OptiPhos Plus at 500 (1× highest recommended level) or 50,000 (100×, group) FTU/kg feed (confirmed by analysis). A positive control group fed a diet supplemented with additional phosphorus was included. The P content in the control and positive control diets were 4.0 and 5.2 g/kg feed in the gestation phase and 4.8 and 6.6 g/kg in the lactation phase, respectively. The diets were mainly based on corn, soya bean meal, sunflower meal and rapeseed meal and administered as pellets. Animals were fed restrictively and according to parity and body condition at mating during gestation and according to the day of lactation and parity during lactation. General health of the animals was monitored throughout the study period. For sows, the feed intake, body weight, back-fat thickness, farrowing performance and interval weaning-to oestrus were measured. For the litters, litter size, birth weight, weaning weight and mortality were registered. Piglets were fed a commercial creep feed from day 7 of life. An ANOVA was done with the data. The model included the round and parity block as random factor and the effects of the dietary treatments as fixed factor. Group means were compared with Tukey's test. Sows and piglets that required medical treatment, mortality of suckling piglets and likely causes of death were analysed using a chi-square test. The significance level was set at $p < 0.05$.

General health conditions of the sows were good during the whole study. No differences were observed in the parameters measured in the sows. The ADFI in gestation was 2.9 kg and in lactation 6.5 kg, the back-fat loss in lactation was 2.3 mm, the mean total piglets born at farrowing was 17 (total born alive 15.2) and the interval weaning-to-oestrus was of 5 days. Piglet's mortality (starting after cross-fostering) was of 15.2% and did not differ among groups and was within the normal range of the experimental farm, a total of 4.4% of the piglets had to receive medication due to different reasons. The average number of piglets weaned per litter was 12 and the body weight of the piglets at weaning was of 8.5 kg.

Feeding the sows with the phytase from OptiPhos® PLUS up to 100-fold the maximum recommended dose did not have any negative effects on the performance parameters on sows or their litters.

Conclusions on the safety for the target species

The FEEDAP Panel concludes that OptiPhos® PLUS is safe for weaned piglets and sows at the recommended level of 500 FTU/kg complete feed with a wide margin of safety. The conclusion is extended to suckling piglets and pigs for fattening. Considering the wide margin of safety shown, the conclusions are extrapolated to all species/categories of Suidae. The solid and the liquid formulations are considered equivalent in terms of safety for the target species.

¹³ Technical dossier/Section II/Annex_III_12.

¹⁴ Technical dossier/Section II/Supplementary information/Annex_RTQ_III_1.2.

3.3. Efficacy

3.3.1. Efficacy for weaned piglets

Trial 1

A total of 36 castrated male piglets (Polish hybrid, 33 day of age, average initial body weight of 9.95 kg) were distributed in individual pens and allocated to one of three dietary treatments (12 replicates per treatment).^{15,16} Two basal diets (pre-starter and starter) based on corn and soybean were either not supplemented (control, pre-starter: P 5.1 g/kg, Ca 5.9 g/kg; starter: P 4.9 g/kg, Ca 5.7 g/kg) or supplemented with OptiPhos® PLUS to provide 250 FTU per kg feed (confirmed by analysis). For each phase, there was a positive control diet (pre-starter: P 6.1 g/kg, Ca 7.5 g/kg; starter: P 6 g/kg, Ca 7.3 g/kg). Diets were offered on *ad libitum* basis as mash form for 42 days. During the last feeding phase of the trial, diets were supplemented with titanium dioxide (TiO₂) as a dietary marker. Mortality and health status were checked every day. Animals were weighed on individual basis on days 1, 21 and 42, feed intake was registered and feed to gain ratio calculated. On days 34, 35, 36, 37 and 38, all faeces were collected daily and pooled per pig for analysis to determine nutrient (dry matter, crude protein, Ca, P) digestibility. Three piglets per treatment were slaughtered on day 42 to sample their third metacarpal bones from the right foot and Ca and P content was determined. An ANOVA was done with the data and considering the pen as the experimental unit. Group means were compared with Tukey's test. The significance level was set at $p < 0.05$.

No animals died during the study. The results are provided in Table 1. The results of the study indicated that the animals receiving phytase at 250 FTU/kg feed had a significantly higher final body weight, better FCR and higher digestibility of P and content of bone ashes.

Trial 2

A total of 18 male piglets (Piètrain × Ra-Se hybrid sow, 4 weeks old, average initial body weight 8.6 kg) were individually housed in metabolic cages and randomly allocated to three dietary treatments (6 replicates per treatment).^{17,18} A basal diet (mainly based on corn, barley, toasted soybeans, wheat, soybean meal) was either not supplemented (control, P 4.8 g/kg and Ca 5.6 g/kg) or supplemented with OptiPhos® PLUS to provide 250 FTU per kg feed (confirmed by analysis). The positive control diet had P 6.3 g/kg and Ca 7.2 g/kg. Silicon dioxide (SiO₂) was added to the diets as a digestibility marker. Diets were offered on *ad libitum* basis as pellets for 6 weeks. Mortality and health status were checked every day. Animals were weighed on day 1, 21, 32, 37 and 42 of the trial. Nutrient (crude protein, Ca, P) digestibility and P retention were determined. On days 33, 34, 35, 36 and 37 all faeces and urine were collected daily and pooled per pig for analysis to determine nutrient (crude protein, Ca, P) digestibility. At the end of the experiment, all the animals were euthanised, and their metacarpus IV (right front leg) was sampled to perform analysis on the ash bone and P and Ca contents. An ANOVA was done with the data and group means were compared with Tukey's test. For the performance data, the model included also the initial body weight as a covariate. The pen was the experimental unit. The significance level was set at $p < 0.05$.

No mortality was observed during the study. One piglet in the positive control group was culled and replaced only to determine P retention. The results are provided in Table 1. No significant differences were observed in the performance of the piglets. The piglets that received the phytase from OptiPhos® PLUS at 250 FTU/kg feed showed compared to the control a higher P retention and a higher bone ash and P content compared to the control.

Trial 3

A total of 192 mixed sex piglets (females and castrated males, Topigs 20 × Piètrain, 28 days of age, average initial body weight 7 kg) were distributed in pens and allocated to one of three dietary treatments (10–11 replicates per treatment, 6 pigs-3 females and 3 castrated males per replicate).¹⁹ Two basal diets (pre-starter and starter) based on wheat, barley, corn, soybean meal and sunflower meal were either not supplemented (control, pre-starter: P 4.3 g/kg, Ca 5.5 g/kg; starter: P 4.6 g/kg,

¹⁵ Technical dossier/Section IV/Annex IV_1.

¹⁶ Technical dossier/Section IV/Annex IV_1_Supplementary Information/Annex_RTQ_IV.1.2.

¹⁷ Technical dossier/Section IV/Annex IV_2.

¹⁸ Technical dossier/Section IV/Supplementary Information_Annex_RTQ_IV.1.3.

¹⁹ Technical dossier/Section IV/Annex IV_2/Supplementary Information (Mar20)/Annex_RTQ_V.162.60.8a.

Ca 6.2 g/kg) or supplemented with the phytase from OptiPhos® PLUS to provide 250 FTU per kg feed (confirmed by analysis). For each phase, there was a positive control diet (pre-starter: P 6.1 g/kg, Ca 7 g/kg; starter: P 6.4 g/kg, Ca 7.7 g/kg). TiO₂ was added to the diets as a digestibility marker. Diets were offered on *ad libitum* basis as pellets for 42 days. Mortality and health status were checked every day. Animals were weighed on individual basis on days 1, 21 and 42, feed intake was registered and feed to gain ratio calculated. From day 31 to 34 representative samples of faeces per each pen were collected daily, to determine nutrient (dry matter, crude protein, Ca, P) digestibility. Faecal material was pooled per pen.

One pig per pen per treatment (median weight) was slaughtered on day 42 to sample metacarpal bones (right front leg) from which perform analysis on the bone ash, Ca and P content was performed. An ANOVA was done with the data and considering the pen as the experimental unit. Group means were compared with the Tukey's test. The significance level was set at $p < 0.05$.

No animals died during the study. The results are provided in Table 1. No significant differences were observed in the feed intake or the final body weight of the piglets. The piglets receiving the phytase from OptiPhos® PLUS at 250 FTU/kg feed showed compared to the control a better feed to gain ratio, higher phosphorus digestibility, and a higher bone ash and phosphorus content compared to the control animals.

Table 1: Effects of OptiPhos® PLUS on the performance of piglets, bone mineralisation and digestibility of phosphorus

	Groups (FTU/kg feed)	Feed intake* (kg)	Weight gain** (kg)	Final body weight (kg)	Feed conversion rate	Bone content***			Digestibility [§]
						Bone ash (%)	P (%)	Ca (%)	P (%)
Trial 1	Control	58.7	26.9 ^b	36.9 ^b	2.20 ^a	29.7 ^b	16.1	32.8 ^b	27.3 ^b
	250	57.4	30.0 ^a	40.0 ^a	1.92 ^b	34.6 ^a	16.5	34.7 ^a	48.4 ^a
	Positive control	57.3	28.6 ^{ab}	38.5 ^{ab}	2.01 ^b	33.3 ^a	16.2	33.2 ^b	40.7 ^a
Trial 2	Control	0.656	0.435	26.8	1.51	31.7 ^b	5.6 ^b	11.9 ^b	54.7 ^b
	250	0.643	0.443	27.2	1.45	35.5 ^a	6.4 ^a	13.5 ^a	62.8 ^a
	Positive control	0.663	0.460	27.9	1.44	34.8 ^a	6.3 ^a	13.3 ^a	60.8 ^a
Trial 3	Control	0.500	0.312	19.8	1.61 ^a	39.8 ^c	7.4 ^c	16.7 ^b	38.2 ^b
	250	0.519	0.344	21.1	1.51 ^b	42.4 ^b	8.0 ^b	17.6 ^b	46.7 ^a
	Positive control	0.522	0.344	21.1	1.53 ^b	45.0 ^a	8.6 ^a	18.7 ^a	45.2 ^a

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

*: Feed intake reported as total feed intake per piglet in trial 1 and as average daily intake per piglet in trials 2 and 3.

**: Weight gain reported as total gain in trial 1 and as average daily gain in trials 2 and 3.

***: In trial 1, Ca and P are reported as % of bone ash.

§: In trial 2, the reported values are for retention.

Conclusions on the efficacy in weaned piglets

The piglets receiving the 6-phytase from OptiPhos® PLUS at the minimum recommended dose of 250 FTU/kg feed showed improvements on the performance in two trials (trials 1 and 3) and on the phosphorus digestibility and bone mineralisation in three trials (with also phosphorus retention in trial 2). The FEEDAP Panel concludes that additive has the potential to be efficacious as a zootechnical additive in weaned piglets.

3.3.2. Efficacy for pigs for fattening

Trial 1

A total of 378 crossbreed male and female pigs (Duroc × (Large White × Landrace), 80 days of age, initial body weight 31.7 ± 5.06 kg) were distributed according to body weight to 42 single-sex

pens in groups of 9 animals.^{20,21} The pens were allocated to three dietary treatments (14 replicates per treatment, 7 replicates with males and 7 replicates with females). Two basal diets (grower and finisher) based on barley, corn, soybean meal and rapeseed meal were either not supplemented (control, total P: grower and finisher 3.9/3.9 g/kg, Ca: 1.3/1.2 g/kg) or supplemented with the phytase from OptiPhos® PLUS to provide 250 FTU per kg feed (confirmed by analysis). For each phase, there was a positive control diet (total P: grower 5.5 g/kg, finisher 5.2 g/kg, Ca: 6.7/6.5 g/kg). TiO₂ was added to the diets as an external marker. The diets were offered on *ad libitum* basis as mash for 90 days. Mortality and health status were checked every day. Animals were weighed on days 80, 116 and 169 days of age (individually), feed intake was registered and feed to gain ratio calculated. Faeces were collected from 135 to 138 days of age and analysed to study nutrient digestibility (including: dry matter, crude protein, ash, organic matter, calcium and phosphorus). An ANOVA was done with the performance data considering the treatment as the main effect, and sex as the fixed effect. Group means were compared with the Bonferroni test. The pen was the experimental unit. The significance level was set at $p < 0.05$.

The overall mortality in the study was 4.6%. The main results are provided in Table 2. The pigs that received the phytase from OptiPhos® Plus at 250 FTU/kg feed showed compared to the control a higher final body weight and ADG, a better FCR, and a higher phosphorus digestibility compared to the control animals.

Trial 2

A total of 45 male hybrid pigs (10 weeks old, initial body weight of 24 kg) were distributed to 15 pens (3 animals per pen) and allocated to three dietary treatments (5 replicates per treatment).^{22,23} Three basal diets (phase 1 from 10 to 15 weeks, phase 2 from 15 to 20 weeks and phase 3 from 20 week to date of slaughter) based on wheat, corn, soybean meal, barley and wheat middlings were either not supplemented (control, phases 1/2/3: P 4.3/4.4/4.2 g/kg, Ca 7.8/6.0/5.5 g/kg) or supplemented with the phytase from OptiPhos® PLUS to provide 250 FTU per kg feed (confirmed by analysis). For each phase, there was a positive control diet (phases 1/2/3: P 5.6/5.6/5.2 g/kg, Ca 7.8/6.9/6.7 g/kg). Acid-insoluble ash was used as a digestibility marker. The trial was divided into 'performance trial' until week 22 of age (45 animals under study for 12 weeks) and a 'balance trial' (two rounds, 9 animals per round taken at week 22 or at week 25). For the balance trial, from 22 to 25 weeks of age, 12 animals were individually housed in metabolic cages. There was a 13 days adaptation period followed by 4 days collection period. Diets were offered on *ad libitum* basis as pellets for 22 weeks of age ('performance trial') and on restricted basis (2.8 times the maintenance needs) during the balance trial. Mortality and health status were checked every day. For the performance evaluation, animals were weighed at 10, 15, 20 and 22 weeks of age (individual), feed intake was registered and feed to gain ratio calculated.

During the balance trial, faecal and urine samples of each pig were collected during four consecutive days. Samples were pooled per pig, and a subsample was taken for analysis. Nutrient (crude protein, Ca, P) digestibility and P retention were determined. Six piglets per treatment were slaughtered at the end of the trial (no details on the selection were given) to sample their metacarpal bones (right front leg) and perform analysis on the bone ash, Ca and P content. An ANOVA was done with the performance data considering the treatment as the main effect, and initial body weight as a covariate. For the balance trial, the diet was considered as a fixed effect and rounds effect as random effect. The test used for group comparison was not reported in the technical dossier. The pen/cage was considered the experimental unit. The significance level was set at $p < 0.05$.

The results are provided in Table 2. The pigs receiving the phytase from OptiPhos® Plus at 250 FTU/kg feed showed compared to the control a higher phosphorus utilisation.

Trial 3

A total of 576 crossbreed male and female pigs (Piétrain x (Large White x Landrace, 77 days old, initial body weight of 25 kg), were blocked according to body weight and sex and distributed to 48 single-sex pens in groups of 12 animals.²⁴ The pens were allocated to three dietary treatments

²⁰ Technical dossier/Section IV/Annex IV_3.

²¹ Technical dossier/Section IV/Supplementary Information/Annex_RTQ_IV.1.4

²² Technical dossier/Section IV/Annex IV_4.

²³ Technical dossier/Section IV/Supplementary Information/Annex_RTQ_IV.1.5.

²⁴ Technical dossier/Supplementary Information/Annex_RTQ_V.4.

(16 replicates per treatment, 8 replicates with males and 8 replicates with females). Two basal diets (grower from day 1 to day 61 and finisher from day 62 to day 83 days of the trial) were either not supplemented (control, total phosphorus: grower and finisher 4 g/kg) or supplemented with the phytase from OptiPhos PLUS® to provide 250 FTU per kg feed (confirmed by analysis). For each phase, there was a positive control diet (total phosphorus: grower 5.8%, finisher 5.4%). Diets were offered on *ad libitum* basis as pellet for 83 days. Mortality and health status were checked daily. Animals were weighed on days 0, 61 and 83 days of trial (individual and pen basis), feed intake was registered (pen basis) and feed to gain ratio calculated. An ANOVA was done with the performance data considering the treatment and sex as fixed effects. Group means were compared with Tukey's test. The pen was the experimental unit. The significance level was set at $p < 0.05$.

The results are provided in Table 2. The pigs receiving the phytase from OptiPhos® PLUS at 250 FTU/kg feed showed better performance compared to the control.

Table 2: Effects of OptiPhos® PLUS on the performance and nutrient digestibility in pigs for fattening

	Groups (FTU/kg feed)	Performance					Utilisation P (%) [*]	Bone mineralisation (%)	
		Average daily feed intake (g)	Average daily gain (g)	Final body weight (kg)	Feed to gain ratio	Mortality (culled) (%)		Ash	P
Trial 1	Control	2,240	791 ^b	102 ^b	2.85 ^a	2.1	18.7 ^b	NA	NA
	250	2,273	864 ^a	109 ^a	2.64 ^b	3.9	28.6 ^a	NA	NA
	Positive control	2,351	893 ^a	112 ^a	2.63 ^b	7.9	30.9 ^a	NA	NA
Trial 2	Control	2,020	879	98	2.30	0	39.8 ^b	57.4 ^b	10.4 ^b
	250	2,180	946	103	2.30	0	49.7 ^a	58.8 ^{ab}	10.7 ^{ab}
	Positive control	2,120	937	103	2.26	0	39.8 ^b	60.1 ^a	10.9 ^a
Trial 3	Control	1,900 ^c	770 ^c	89.4 ^c	2.46 ^a	3.13 (5.73) ^a	NA	NA	NA
	250	2,050 ^b	870 ^b	96.9 ^b	2.37 ^b	3.13 (2.60) ^a	NA	NA	NA
	Positive control	2,190 ^a	940 ^a	102.3 ^a	2.32 ^b	0.52 (0.52) ^b	NA	NA	NA

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

NA: not analysed.

*: In trial 1, the reported values are for digestibility.

Conclusions on the efficacy in pigs for fattening

The pigs for fattening receiving the phytase from OptiPhos® PLUS at the minimum recommended dose of 250 FTU/kg feed showed improvements on the performance in two trials (trials 1 and 3) and on the phosphorus utilisation in one trial (trial 2) compared to the control group. The FEEDAP Panel concludes that additive has the potential to be efficacious as a zootechnical additive in pigs for fattening.

3.3.3. Efficacy for sows

A total of four efficacy studies were submitted in which the sows and litters were either studied during the late-gestation and lactation phase. Data on the effect of the additive on the apparent faecal digestibility of phosphorus was provided in the gestation phase (trials 3 and 4) and lactation phase (trials 1–4).

Trial 1

A total of 40 Danbred sows (parity number between 2 and 6), from two batches, were under study from 2 weeks before the expected farrowing date until weaning of piglets (day 24 of age).²⁵ The sows

²⁵ Technical dossier/Section IV/Annex IV_5.

were allocated to the 4 treatments, representing 10 replicates per treatment. The basal diets (gestation and lactation) based on barley, maize, soybean meal, wheat gluten feed and beet pulp (plus sunflower meal during gestation) were either not supplemented (control: P 4.7 and Ca 7.0 g/kg in gestation; 4.8 and 7.5 in lactation) or supplemented with the phytase from OptiPhos® PLUS to provide 250 or 500 FTU/kg (confirmed by analysis. A positive control was also included (P 6.5 and Ca 9.0 g/kg feed in gestation; and 6.6 and 9.5 in lactation). During gestation, and until 2 days after farrowing, sows received 3 kg feed per day thereafter feed administration was increased up to a maximum of 7.5 kg/sow per day. The feed was offered to sows in pelleted form. General health and mortality of the animals was monitored daily. The parameters measured in sows included the feed intake, body weight and back-fat thickness loss during lactation and farrowing performance parameters. Cross-fostering of piglets was done within treatment during the first 48 h of life to equalise the litters to 13 piglets and the body weight of the piglets was registered. Piglets received a commercial creep feed from 7 days of age and were weaned on day 24 of age. At that point, the number of piglets per litter and the individual body weight were measured. The daily intake of creep feeding of each litter was measured. On the last week of lactation, faeces from sows were collected for 5 days to measure apparent faecal digestibility coefficients of P, Ca, crude protein and phytate.²⁶ Acid-insoluble ash concentration was used as a marker in feed and excreta. An ANOVA was done with the diet as the fixed effect and the round and parity as random effects. Mean groups were compared with Bonferroni test. Mortality was analysed with Kruskal–Wallis test. The significance was set at $p \leq 0.05$.

The results of the main parameters are presented in Table 3. The sows receiving the phytase from OptiPhos® PLUS at any level showed, compared to the control group, a significantly higher apparent faecal phosphorus digestibility. No other significant differences were identified in any of the parameters studied except for a significant difference in the number of piglets per litter at weaning, which was significantly lower in the 250 FTU/kg feed compared to control.

Trial 2

A total of 51 Large White x Landrace sows (parity number between 2 and 8), from two batches, were under study from two weeks before the expected farrowing date until weaning of piglets (day 28 of age). The sows were allocated to 4 treatments, representing 13 or 12 replicates per treatments.²⁷ Basal diets (gestation and lactation) based on maize, soybean meal, sunflower meal and beet pulp was either not supplemented (control: P 4.3 and Ca 5.8 g/kg in gestation and 4.6 and 5.3 in lactation) or supplemented with the phytase from OptiPhos® Plus to provide 250 or 500 FTU/kg feed. A positive control was also included (P 6.1 and Ca 7.8 g/kg in gestation and P 6.4 and Ca 7.3 in lactation). Feeding of sows was restricted during gestation and after farrowing the amount of feed provided to the sows was gradually increased in order to reach *ad libitum* level. Feed was offered in pelleted form. General health and mortality of the animals was monitored daily. The parameters measured in sows included the feed intake, body weight loss during lactation and farrowing performance parameters. Cross fostering of piglets was done in the first 3 days of life, and piglets received creep feeding from day 14 of life. Piglets and litter weight were recorded at birth, 7 days post-farrowing and weaning. On the last week of lactation and for 4 consecutive days, faeces from sows were collected to determine apparent digestibility coefficients of P, Ca, ash and dry matter by using TiO₂ as a digestibility marker. The data were analysed by ANOVA (with treatment as fixed effect and batch as the random effect) and group means were compared with Tukey's test. The sow with its respective litter was the experimental unit for all analyses. Number of piglets per litter at different time points and piglets' mortality was analysed with non-parametric methods. The significance was set at $p \leq 0.05$.

The results of the main parameters are presented in Table 3. The sows receiving the phytase from OptiPhos® PLUS showed compared to the control group a significantly higher apparent faecal phosphorus digestibility from the 250 FTU/kg feed. No other significant differences were identified in any of the parameters studied except for a significant difference in the number of piglets per litter at weaning, which showed a significantly lower litter size in the 250 FTU/kg feed compared to control.

Trial 3

A total of 51 Landrace x Large White sows (parity from 1 to 8), from three batches, were under study from day 35 before the expected farrowing date until weaning of piglets (day 21 of age).²⁸ The sows were allocated to 3 dietary treatments, representing 17 replicates per treatment. Basal diets

²⁶ The values for phytate digestibility showed values extremely high and were not considered further.

²⁷ Technical dossier/Section IV/Annex IV_6.

²⁸ Technical dossier/Section IV/Supplementary data_Annex V_2.

based on barley and soybean meal (plus sunflower meal during gestation) were either not supplemented (control: P 4.7 and Ca 6.8 g/kg feed in gestation and P 4.6 and Ca 7.5 g/kg feed during lactation) or supplemented with OptiPhos® Plus G to provide 250 FTU/kg feed (confirmed by analysis). A positive control was also included (P 6.8 and Ca 8.8 g/kg feed in gestation and P 6.6 and Ca 9.5 in lactation). During the gestation sows received 2.94 kg feed per day and during the lactation the feed allowance was increased according to individual sows' requirements. Feed was offered in pelleted form. General health and mortality of the animals was monitored throughout the study. The parameters measured in sows included the feed intake, body weight and back-fat loss during lactation and farrowing performance parameters. Cross fostering of piglets was done in the first day of life, and piglets did not receive creep-feed during the lactation. Piglets' weight was measured at birth, after cross-fostering and at weaning. Faeces from the sows were collected for 4 days in the gestation (from day 14 to 10 before farrowing) and in the lactating phase (from day 17 to 21 days after farrowing). Samples were pooled per sow within each phase (gestation or lactation). Afterwards, they were analysed for dry matter, crude protein, phytic P, phosphorus and calcium (34 samples in total per treatment) using TiO₂ as a digestibility marker. Data were analysed by GLM (with the treatment as the fixed effect) and group means were compared with Tukey's test. The sow with its respective litter was the experimental unit for all analyses. The significance was set at $p < 0.05$.

The results of the main parameters are presented in Table 3. The sows receiving the phytase from OptiPhos® PLUS showed compared to the control group a significantly higher apparent faecal phosphorus digestibility (only in the gestation) and phytate digestibility (gestation and lactation) at 250 FTU/kg feed. No other significant differences were identified in the parameters studied except for a lower feed intake of the sows in the group receiving 250 FTU/kg feed which had no impact in any of the parameters studied.

Trial 4

A total of 37 hybrid sows (parity from 1 to 9), from two batches were under study from day 38 before the expected farrowing day to day 35 of lactation.²⁹ The sows were allocated to 3 dietary treatments (taking into account parity and bodyweight of the sows at the start of the trial), representing 12 or 13 replicates per treatment. Basal diets based on maize, barley, soybean meal and beet pulp was either not supplemented (control: P 4.2 and Ca 7.8 g/kg feed in gestation and P 4.6 and Ca 6.8 g/kg feed in lactation) or supplemented with OptiPhos® Plus G to provide 250 FTU/kg feed (confirmed by analysis). A positive control was also included (P 5.9 and Ca 7.8 g/kg feed in gestation and P 6.1 and Ca 8.8 in lactation). Until day 107 of gestation the sows were kept in groups and then were moved to the lactation barns where they were housed individually. During the gestation sows received 2.6 kg feed per day and when sows were transferred to the farrowing barns the amount of feed was increased until 3.0 kg per day, and after farrowing the amount was increased progressively and individually until 7.5 kg/day. Feed was offered restricted in pelleted form. General health and mortality of the animals was monitored throughout the study. The parameters measured in sows included body weight and back-fat loss during lactation and farrowing performance parameters measured on day 28 of lactation. Cross fostering of piglets was done within the first 24 h of life and piglets received creep-feed from day 7 of life. Piglets weight was measured at birth, after cross-fostering and at day 28 of lactation.

Faecal samples were collected rectally from all sows for 4 days in gestation in the 2 weeks before farrowing and in the fifth week of lactation. Faecal samples were pooled per sow within the same sampling period (lactation or gestation). The total apparent faecal digestibility of dry matter, crude ash, crude protein, P and Ca using acid-insoluble ash as a marker. To analyse the different parameters, a linear mixed model, followed by Tukey's test, was used with the diet as fixed factor and round as random effect. To analyse the piglets' body weight at birth and at start, the number of piglets at birth was added to the model as a covariate. The same approach was used for piglet parameters at weaning (the number of piglets at weaning was added to the model as a covariate). The significance was set at $p < 0.05$.

The results of the main parameters are presented in Table 3. The sows receiving the phytase from OptiPhos® PLUS showed compared to the control group a significantly higher apparent faecal phosphorus digestibility (both during gestation and lactation) at 250 FTU/kg feed. No other significant differences were identified in the parameters studied. The FEEDAP Panel notes that the feed intake of the sows was not given in the study report, only the allowances.

²⁹ Technical dossier/Section IV/Supplementary data_Annex V_3.

Conclusions on the efficacy in sows

The sows that received the additive at 250 FTU/kg feed showed improvements on the apparent faecal phosphorus digestibility during gestation in two trials (trials 3 and 4) and during lactation in three trials (1, 2 and 4). In trial 3, the digestibility of phytate was higher in sows that received the additive at 250 FTU/kg. The FEEDAP Panel concludes that additive has the potential to be efficacious as a zootechnical additive in sows.

Table 3: Effects of Optiphos® Plus on the feed intake and body condition of sows and litter performance during the lactation, and on the apparent faecal digestibility of phosphorus and digestibility of phytate in the gestation/lactation phase

	Group (FTU/kg feed)	Sows' parameters in lactation			Faecal digestibility in sows		Litter size (n)		Piglets' weight (kg)		Piglets' mortality/culls (% or n) ³
		Daily feed intake (g)	Body weight loss ¹	Back-fat loss	P apparent digestibility (%) ²	Phytate digestibility (%) ²	Initial	Final	Initial	Final	
Trial 1	Control	5,212	15.5	2.9	NA/25.7 ^c		13.0	12.7	1.58	6.33	2.3
	250	5,325	14.6	2.1	NA/36.1 ^b		13.0	12.1	1.68	6.44	6.2
	500	5,351	15.8	2.4	NA/45.2 ^a		13.0	12.9	1.68	6.51	1.7
	Positive control	5,316	14.0	2.1	NA/24.3 ^c		13.0	12.5	1.62	6.47	3.8
Trial 2	Control	4,830	1.22	–	NA/36.2 ^c		14.1	13.1 ^a	2.62	6.96	0.9
	250	4,750	0.87	–	NA/46.3 ^{ab}		12.9	10.7 ^b	2.56	7.14	2.2
	500	4,790	1.13	–	NA/49.4 ^a		12.3	11.5 ^{ab}	2.58	7.47	0.8
	Positive control	4,460	1.18	–	NA/43.9 ^b		11.8	10.9 ^{ab}	2.76	7.79	0.8
Trial 3	Control	6,310 ^a	26.0	1.47	27.7 ^c /30.6	60.9 ^b /73.1 ^b	11.6	9.9	1.68	6.06	13.2
	250	5,680 ^b	31.5	1.06	45.2 ^a /32.1	81.7 ^a /88.0 ^a	10.8	9.6	1.71	6.15	9.6
	Positive control	6,000 ^{ab}	29.5	0.71	40.2 ^b /32.8	57.3 ^b /77.2 ^b	11.4	10.1	1.65	6.46	11.1
Trial 4	Control	–	47	5.3	29.8 ^b /43.1 ^b		13.2	11.4	1.50	8.50	12.7
	250	–	36	5.6	34.7 ^a /46.9 ^a		13.3	12.1	1.50	8.50	7.6
	Positive control	–	47	5.8	35.2 ^a /42.4 ^b		12.8	11.3	1.40	8.60	11.0

¹: Body weight loss in trials 1, 3 and 4 expressed as total kg loss, in trial 2 expressed in kg loss/day.

²: Values in trials 3 and 4 are for gestation/lactation phase, respectively.

³: Percent values in trials 1, 3 and 4 and n values for trial 2.

a,b,c: Within a trial, values within a column with different superscript are significantly different ($p < 0.05$).

NA: not analysed.

3.3.3.1. Conclusions on efficacy

Based on the results of the studies, the FEEDAP Panel concludes that additive has the potential to be efficacious as a zootechnical additive in weaned piglets, pigs for fattening and sows at the level of 250 FTU/kg feed. The conclusion in weaned piglets is extended to include suckling piglets. Since the mode of action of phytases is well-known and can reasonably be assumed to be the same between porcine species the FEEDAP Panel extrapolates the conclusions to other minor porcine 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 Regulation³⁰ and Good Manufacturing Practice.

³⁰ 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.

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 suckling and weaned piglets, pigs for fattening, sows, other minor pig species for fattening and other minor reproductive pig species at the maximum 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 suckling and weaned piglets, pigs for fattening, sows, other minor pig species for fattening and other minor reproductive pig species 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
28/08/2019	Application validated by EFSA – Start of the scientific assessment
24/04/2019	Comments received from Member States
25/11/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
11/12/2019	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>
02/03/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
01/07/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ANOVA	analysis of variance
bw	body weight
CAS	Chemical Abstracts Service
CV	coefficient of variation
EURL	European Union Reference Laboratory
FCR	feed conversion rate
FEEDAP Panel	EFSA Panel on Additives and products or Substances used in Animal Feed
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.