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## Safety and efficacy of OptiPhos<sup>®</sup> PLUS (6 phytase) for laying hens, turkeys for breeding, chickens for breeding, minor poultry species for egg production purposes and breeding

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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 the safety and efficacy of OptiPhos PLUS<sup>®</sup>, intended to be used as a feed additive for laying hens, turkeys for breeding, chickens for breeding, minor poultry species for egg production purposes and breeding. The active substance is 6-phytase produced by a genetically modified strain of *Komagataella phaffii*. The EFSA FEEDAP Panel concluded that the genetic modification of the production strain does not give rise to safety concerns. 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. The additive was considered safe also for the consumer and the environment. 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 concluded that the additive has the potential to be efficacious in increasing the phosphorus utilisation in laying hens at the level of 250 FTU/kg feed. These conclusions were extended to turkeys for breeding and chickens for breeding and extrapolated to minor poultry species for egg production purposes and breeding.

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**Keywords:** OptiPhos PLUS<sup>®</sup>, laying hens, *Komagataella phaffii*, safety, efficacy, QPS, extrapolation

**Requestor:** European Commission

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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 laying hens, turkeys for breeding, chickens for breeding, minor poultry species for egg production purposes and breeding (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 7 November 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 an opinion on OptiPhos® PLUS (6-phytase) produced by a genetically modified strain of *Komagataella phaffii* (DSM 32854) for the use as a feed additive in poultry species for fattening, minor poultry species reared for breeding and ornamental birds (EFSA FEEDAP Panel, 2020).

## 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, 2012), 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,

<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-2019-0052.

<sup>4</sup> The full report is available on the EURL website: [https://ec.europa.eu/jrc/sites/jrcsh/files/finrep\\_fad-2019-0052\\_optiphos\\_plus\\_egg-producing.pdf](https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2019-0052_optiphos_plus_egg-producing.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.

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 and is proposed to be used as zootechnical additive (functional group: digestibility enhancers) for laying hens, turkeys for breeding, chickens for breeding, minor poultry species for egg production purposes and breeding.

#### 3.1. Characterisation

The additive is produced by a genetically modified strain of *K. phaffii* (previously *P. 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 the previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2020).

The additive is intended to be used in feed for laying hens, chickens for breeding purposes, turkeys for breeding purposes, minor poultry species for egg production purposes and minor poultry species for breeding purposes at a minimum inclusion level of 250 FTU/kg of complete feedingstuff. The recommended inclusion level ranges from 250 to 500 FTU/kg feedingstuff.

#### 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, 2020). The Panel concluded that 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 laying hens

The applicant provided two tolerance studies that followed the same trial design.<sup>6,7</sup> In each study, a total of 675 17-week-old hens (Dekalb white) were distributed in 45 pens of 15 hens each. Birds were fed a commercial diet for three weeks. During this acclimatisation period, their early laying performance was monitored. In the first study, an outbreak caused by *Eimeria tenella* and necrotic enteritis occurred during the acclimatisation period and required inclusion in the feed of a coccidiostat (amprolium at 20 mg/kg) and a macrolide (acetyl isovaleryl tylosin at 25 mg/kg) for 5 days (from D10 to D15). Since the treatment was finished at the start of the study and there were no more signs of disease, the FEEDAP Panel considers that the treatment will have no relevant effect on the study. However, some of the hens died and therefore some replicates presented 14 hens instead of 15. In each study, at the age of 20 weeks, three treatments were allocated to the replicates (15 replicates per treatment of 14/15 hens each). A basal diet based on maize, sunflower meal and soybean meal, was either not supplemented (control) or supplemented with OptiPhos® PLUS to provide 500 (1× maximum recommended dose) or 50,000 (100× maximum recommended dose) FTU per kg feed (confirmed by analysis). The basal diet contained 5.8 g/kg total phosphorus, 3.2 g/kg digestible

<sup>6</sup> Technical dossier/Section III/Annex\_1.

<sup>7</sup> Technical dossier/Section III/Supplementary Information\_Annex III\_1.3.

phosphorus, and 37 g/kg calcium. The feed was offered in mash form and restricted (100 g D0–D28 and 110 g D28–D56) for 56 days.

Mortality and general health were monitored throughout the study. Body weight per replicate was recorded at the beginning and at the end of the trial. Feed consumption was recorded at day 28 and day 56 per pen. Egg production per pen was recorded and weighed every second day. Feed to egg mass ratio was calculated. For the statistical analyses, the pen was considered as the experimental unit. An analysis of variance (ANOVA) was done with the performance data and considering the treatment as the fixed effect. Group means were compared with Tukey's test. The significance level was set at  $p \leq 0.05$ .

Mortality was 0.45% and 0.14% for the first and second study, respectively, and no differences were found between treatments. No significant differences between treatments were observed in egg production, daily egg mass, body weight, daily weight gain, daily feed intake, feed conversion ratio and feed to egg production ratio (control group values average daily feed intake was 105.5/103.2 g, laying rate was 84.3/87.7%, daily egg mass 44/46.1 g/bird and feed to egg mass ratio was 2.41/2.24). In trial 1, shell weight was significantly lower in the 1× group compared to the control (7.90 vs. 8.08 g), but shell thickness was significantly increased in the treated groups compared to the control and no differences were seen in shell strength. In the second study, the average shell thickness was significantly higher in the group supplemented additive with 50,000 FTU/kg of OptiPhos® PLUS compared to control.

Feeding the birds with the OptiPhos® PLUS up to 100× the recommended dose did not have any negative effects on the performance parameters (average body weight, average daily weight gain, average daily feed intake, feed conversion rate).

### 3.2.1.2. Conclusions on safety for the target species

The FEEDAP Panel concludes that OptiPhos® PLUS is safe for laying hens at the recommended level of 500 FTU/kg complete feed with wide a margin of safety. This conclusion is extended to turkeys for breeding, chickens for breeding, minor poultry species for egg production purposes and breeding. The solid and the liquid formulations of the additive are considered equivalent in terms of safety for the target species.

## 3.3. Efficacy

### 3.3.1. Efficacy for laying hens

Three balance trials, done in laying hens were submitted for the assessment. Two trials (1 and 2), which also included performance, shared a similar experimental design.

#### **Trials 1 and 2**

In the first trial, a total of 176 Isa Brown hens of 25 weeks of age were distributed in 88 cages of 2 hens each.<sup>8</sup> In the second trial, a total of 192 Hy-line brown hens of 19 weeks of age were distributed in 96 cages of 2 hens per cage.<sup>9</sup> From 17 to 25 weeks of age (in the first trial) and from 19 to 22 weeks of age (in the second trial), the animals were fed a pre-laying commercial diet without enzyme additive (acclimatisation period). In the first trial, at 25 weeks of age, they were allocated to 4 dietary treatments (22 replicates per treatment). A basal diet based on corn and soybean meal, containing low phosphorus concentration (negative control; 0.43% total P; 0.18% non-phytate P and 3.63% Ca) was either not supplemented or supplemented with OptiPhos® PLUS at 250 or 500 FTU/kg feed (confirmed by analysis). A fourth treatment group containing the same basal diet but with normal content of phosphorus (0.55% total P; 0.3% non-phytate P and 3.63% Ca) constituted the positive control. In the second trial, at 23 weeks of age they were allocated to three dietary treatments (32 replicates per treatment) balanced on laying rate during acclimatisation period. A basal diet based on maize and soybean meal with low phosphorus content (4.57 g P/kg feed and 41.6 g Ca/kg feed) was not supplemented with the additive (negative control) or supplemented with OptiPhos® PLUS at 250 FTU/kg feed (confirmed by analysis). The basal diet described above containing appropriate levels of phosphorus (5.72 g P/kg feed and 37.8 Ca/kg feed) constituted the experimental diet for the positive control group.

<sup>8</sup> Technical dossier/Section\_IV/Annex\_1.

<sup>9</sup> Technical dossier/Section\_IV/Annex\_3.

The feed was offered in mash form *ad libitum* for 84 days. Titanium dioxide was added to the experimental diets as a marker for digestibility studies.

Mortality and general health were monitored throughout the study. Body weight per replicate (cage) was recorded at the beginning and at the end of the trial. Feed consumption was recorded for trial 1 at day 28, day 56 and day 84 per cage, and for trial 3 every 4-week period of trial. Egg production per pen was recorded and eggs were weighed. Feed to egg mass ratio was calculated.

For each treatment, two eggs from 12 randomly selected cages (from a total of 22 cages for trial 1 and 32 cages for trial 2) were collected at the end of trial. Tibia ash were collected at end of trial on 48 animals for trial 1 (12 cages per treatment, the left tibias of both hens per cage were pooled) and on 36 animals for trial 2 (6 cages per treatment, the left tibias of both hens per cage were pooled). Excreta samples (100 g) were collected daily in two trays per replicate (10 replicates of 4 birds/treatment) at 42–45 days on trial. After finishing the collection period, excreta were pooled per each replicate, then excreta samples were dried and analysed for marker, phosphorus, dry matter and ash.

Statistical analyses were performed by ANOVA and the cage was the experimental unit. When differences were encountered, the *post hoc* Tukey's test was used to determine differences between the treatment groups. The significance level was set at  $p \leq 0.05$ .

No hens died during the studies. No significant differences were observed on performance parameters when comparing the groups treated with the additive with the negative control group.

Results are reported in Table 1. There was no effect of the additive on the egg phosphorus content. There was a positive significant effect of the additive at 250 FTU/kg feed on the tibia dry matter content (trial 1) and a positive significant effect of the additive at doses 250 (trials 1 and 2) and 500 (trial 2) FTU/Kg feed on the tibia ash. Similar effects were seen on calcium and phosphorus contents in tibia for the animals in trial 1. There was also a significant positive effect of the additive on phosphorus utilisation at 250 (trials 1 and 2) and at 500 (trial 1) FTU/kg feed.

**Table 1:** Effects of OptiPhos® PLUS on the bone mineralisation and P content and P utilisation in laying hens (trials 1 and 2)

Trial	Groups (FTU/kg feed)	Bone mineralisation (d 84)				Egg content	Utilisation
		DM (%)	Tibia ash (%DM)	Ca (%DM)	P (%DM)	P (%DM)	P (%)
Trial 1	Negative control	59.97 <sup>b</sup>	47.97 <sup>b</sup>	18 <sup>c</sup>	8.68 <sup>b</sup>	1.08	25.18 <sup>b</sup>
	250	64.8 <sup>a</sup>	50.54 <sup>a</sup>	19 <sup>ab</sup>	9.25 <sup>a</sup>	1.13	28.75 <sup>a</sup>
	500	61.77 <sup>ab</sup>	52.06 <sup>a</sup>	19.85 <sup>a</sup>	9.45 <sup>a</sup>	1.13	30.90 <sup>a</sup>
	Positive control	62.95 <sup>ab</sup>	49.93 <sup>ab</sup>	18.81 <sup>bc</sup>	9.05 <sup>ab</sup>	1.15	23.98 <sup>b</sup>
Trial 2	Negative control	53.35	47.34 <sup>b</sup>	18.57	9.05	0.562	30.47 <sup>b</sup>
	250	58.04	51.01 <sup>a</sup>	19.48	9.66	0.577	34.47 <sup>a</sup>
	Positive control	57.42	49.18 <sup>ab</sup>	19.05	9.2	0.567	34.26 <sup>a</sup>

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

DM: dry matter.

### Trial 3

A total of 36 Lohmann Brown hens of 28 weeks of age were caged individually in battery cages.<sup>10,11</sup> From 28 to 30 weeks of age (monitoring period), all animals were fed a basal diet consisting of maize, soybean meal and sunflower meal with adequate phosphorus content (analysed 0.6% P and 0.4% Ca) without the additive (positive control diet) and laying rate was monitored. At 30 weeks of age, hens were allocated to three dietary treatments (12 replicates of 1 bird per treatment) balancing for laying performance. One treatment group consisted in the positive control diet described above. The other treatment groups received the basal diet described above but with low phosphorus content (analysed 0.45% P and 0.39% Ca) which was either not supplemented (negative control) or supplemented with OptiPhos PLUS® at 250 FTU/kg feed (confirmed by analysis). Titanium dioxide (0.5%) was added to the diets as an external marker. The experimental diets were offered in mash form and *ad libitum* for 6 weeks (adaptation period), during this period individual body weight, feed intake, laying rate and egg

<sup>10</sup> Technical dossier/Section\_IV/Annex\_2.

<sup>11</sup> Technical dossier/Section\_IV/Supplementary Information\_Annex\_RTQ\_IV.2.

weight were monitored. Subsequently, a balance study was done with a collection period of 4 days. Total phytic phosphorus, calcium, crude protein and titanium in feed and in excreta were measured. Tibia bones were collected from 8 birds/treatments to determine ash, P and Ca content; total P content of whole eggs laid during the digestibility period was also measured. Mortality and general health were monitored throughout the study. An ANOVA was performed on the data obtained and when differences were detected, mean differences between groups were compared by Tukey test. Non-parametric Kruskal–Wallis test was used when variances were heterogeneous on cage basis. The significance level was set at  $p \leq 0.05$ .

**Table 2:** Effects of OptiPhos® PLUS on the bone mineralisation and P content and P utilisation in laying hens (trial 3)

Groups (FTU/kg feed)	Bone mineralisation			Utilisation	Digestibility	Egg content (Fresh matter)	
	Tibia ash (%DM)	Ca (%DM)	P (%DM)	Total P (%)	Phytate P (%)	Ash (%)	P (%)
Negative control	56.55	18.66	8.52	9.3	62.6 <sup>b</sup>	10.2	0.095 <sup>a</sup>
250	56.33	18.27	8.65	21.4	84.6 <sup>a</sup>	10.17	0.093 <sup>b</sup>
Positive control	57.58	18.9	8.22	12.3	62.8 <sup>b</sup>	10.23	0.10 <sup>a</sup>

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

One animal from the negative control group died during the sixth week of the adaptation period. No differences in body weight, body weight gain, feed intake, feed to gain ratio, laying rate, egg weight, egg mass/hen per day or feed to egg mass ratio were observed.

Results are reported in Table 2. The additive had no effect on bone mineralisation but significantly ( $p \leq 0.05$ ) increased the digestibility of phytate phosphorus and a tendency ( $p = 0.074$ ) to increase total phosphorus utilisation was observed.

### 3.3.1.1. Conclusions on efficacy

The inclusion of the additive in the diet of laying hens increased the total phosphorus utilisation coupled with increased bone ash content in two trials (1 and 2) and phytate phosphorus digestibility in another trial (3).

The FEEDAP Panel concludes that the additive has the potential to be efficacious in increasing the phosphorus utilisation in laying hens at the level of 250 FTU/kg feed.

The conclusions on laying hens can be extended to turkeys for breeding and to chickens for breeding at the corresponding dose.

The mode of action of the phytase is well known and can be considered similar in all poultry/avian species. Therefore, the conclusions drawn in laying hens can be extrapolated to minor poultry species for egg production purposes and breeding.

## 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>12</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 laying hens, turkeys for breeding, chickens for breeding and in minor poultry species for egg production purposes and breeding 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.

<sup>12</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 268, 8.2.2003, p. 1.



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 laying hens, turkeys for breeding, chickens for breeding and in minor poultry species for egg production purposes and breeding 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
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>
06/02/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
11/02/2020	Comments received from Member States
02/03/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
25/05/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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## Abbreviations

ANOVA	analysis of variance
DM	dry matter
EURL	European Union Reference Laboratory
FCR	feed conversion ratio
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
QPS	Qualified presumption of safety

## 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 avian species for egg production and/or breeding purposes 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 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 named 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 colorimetric standard methods EN ISO 30024 (for *feedingstuffs*) and VDLUFA 27.1.4 (for the *feed additive*). In addition, the Applicant applied with minor experimental modifications also the VDLUFA 27.1.4 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 colorimetric 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.