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Safety and efficacy of OPTIPHOS[®] (6-phytase) as a feed additive for finfish

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Abstract

The additive OPTIPHOS[®] is a preparation of 6-phytase that is authorised for use in avian species, weaned piglets, pigs for fattening and sows. The applicant seeks for an extension of use of the product to finfish, at a dose range between 250 and 1,000 OTU/kg feed. The FEEDAP Panel concluded in a previous opinion that there are no concerns for consumer safety and no risks for the environment are expected from the use of the product as feed additive. Moreover, it was concluded that the additive is not a skin/eye irritant or a skin sensitiser, but has the potential to be a respiratory sensitiser. The Panel considered that the new use of the additive would not change the previous conclusions regarding the safety for the consumer, user and environment. The results of a tolerance study performed in juvenile rainbow trout (*Oncorhynchus mykiss*) showed no negative effects of the additive on the fish when offered up to 100 times the maximum recommended dose. Therefore, the Panel concluded that the additive is safe for the rainbow trout and extrapolated this conclusion to all finfish. The Panel evaluated three efficacy trials, two performed in rainbow trout and one in Atlantic salmon (*Salmo salar*). In these studies, the performance of the fish was monitored as well as phosphorus digestibility and phosphorus retention parameters. In the three trials, the performance and the phosphorus retention were improved by the additive at the lowest tested dose (in rainbow trouts at 250 OTU/kg feed and in Atlantic salmon at 500 OTU/kg feed). The FEEDAP Panel concluded that the additive has a potential to be efficacious in rainbow trout and salmon at 500 OTU/kg. The Panel extrapolated the conclusion to all finfish species.

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1. Introduction

1.1. Background and Terms of Reference

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® (6-phytase), when used as a feed additive for all finfish (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 20 June 2016.

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® (6-phytase), when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

The additive OPTIPHOS® is a preparation of 6-phytase produced by a genetically modified strain of *komagataella pastoris* (DSM 23036; formerly known as *Pichia pastoris*). This additive is authorised for use in avian species, weaned piglets, pigs for fattening and sows.

The Panel on Additives and Products or Substances in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of OPTIPHOS® (6-phytase) as a feed additive for avian and porcine species (EFSA FEEDAP Panel, 2011b) and another one on the modification on the terms of the authorisation for pigs for fattening (EFSA FEEDAP Panel, 2015). These opinions considered the safety aspects of the additive regarding the consumer, the user, the environment and the genetic modification of the production strain.

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® as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008⁴ and the applicable EFSA guidance documents.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁵

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of OPTIPHOS® is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011a), Guidance for

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, 1113 SOFIA, Bulgaria.

³ FEED dossier reference: FAD-2016-0019.

⁴ 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.

⁵ The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finirep_fad-2016-0019_optiphos.pdf

establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c) and Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008).

3. Assessment

The additive OPTIPHOS® is a preparation of 6-phytase produced by a genetically modified strain of *K. pastoris* (DSM 23036; formerly known as *P. pastoris*). The additive is authorised for use in avian species, weaned piglets, pigs for fattening and sows. The applicant seeks for an extension of use of the product to all finfish.

3.1. Characterisation

3.1.1. Characterisation of the additive

The assessment of the production strain, manufacturing process as well as the resulting formulations were described in detail in a previous opinion (EFSA FEEDAP Panel, 2011b). OPTIPHOS® is available in two solid formulations (OPTIPHOS® G 4000 and OPTIPHOS® CT 4000) and in a liquid formulation (OPTIPHOS® L 8000). The minimum phytase activity in the solid formulations is of 4,000 OTU⁶/g and in the liquid is of 8,000 OTU/g.

3.1.2. Stability

The applicant provided new data on the shelf-life of the solid formulations and on the stability of the liquid formulation in feed for fish.

The shelf-life of the solid formulations was studied in three batches for each form; the initial mean enzyme activity was 4,426 OTU/g for the CT form and 4,466 OTU/g for the G form. Samples were kept in a package consisting of primary polyethylene bag and secondary paper back at 25°C for 18 months or at 37°C for 6 months.⁷ After 18 months at 25°C, mean recoveries of the initial enzyme activity were 97% and 92% for the CT and G formulation, respectively. After 6 months at 37°C, mean recoveries were 91% and 87% for the CT and G formulation, respectively.

The stability of the phytase in feed for fish was studied using three batches of the liquid formulation (L).⁸ The additive was spray-dried in a total of six batches of a complete diet at either 500 or 750 OTU/kg feed. Samples of each diet were kept in closed containers at 15 or 25°C for 12 weeks or at 37°C for 16 weeks. Mean recovery of the initial enzyme activity after 12 weeks of storage at 15°C was of 91%, at 25°C was of 74% and at 37°C was of 77%. Samples stored at 37°C for 16 weeks showed mean recoveries of 65%. Ten subsamples of each feed were analysed to study the capacity of the phytase to homogeneously distribute. The coefficient of variation shown was below 6%.

3.1.3. Conditions of use

The additive is to be used in feed for finfish at a minimum recommended dose of 250 OTU/kg feed and a maximum recommended dose of 1,000 OTU/kg feed.

3.2. Safety

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 users and for the environment have been previously evaluated (EFSA FEEDAP Panel, 2011b). The FEEDAP Panel concluded that there are no concerns for consumer safety and no risks for the environment are expected. Considering the safety for the user, it was concluded that the additive is not an irritant to skin or eye and is not a skin sensitiser. Owing to the proteinaceous nature of the active substance, the additive has the potential to be a respiratory sensitiser.

⁶ One unit of phytase activity (OTU) is defined as the amount of enzyme that catalyses the release of 1.0 µM of inorganic phosphate per minute from 5.1 mM sodium phytate in pH 5.5 citrate buffer at 37°C, measured as the blue P-molybdate complex colour at 820 nm.

⁷ Technical dossier/Section II/Annexes II.43 and II.44.

⁸ Technical dossier/Section II/Annex II.58.

The FEEDAP Panel is not aware of any new information that would lead it to reconsider the conclusions drawn previously. Moreover, the FEEDAP Panel considers that the new use requested by the applicant would not modify the above conclusions.

Since the application covers the use of the additive in fish, the applicant provided one tolerance study in rainbow trout.

3.2.1. Safety for the target species

In a combined tolerance and efficacy trial, a total of 980 juvenile rainbow trout (*Oncorhynchus mykiss*) were reared in standard conditions for approximately 3 months. Afterwards, with a body weight of 21.5 ± 0.8 g fish were distributed in groups of 35 to 28 tanks and allocated to seven dietary treatments (four replicate tanks per treatment).⁹ A basal diet based on fish and krill meal, soya and pea protein concentrate, guar meal, wheat, corn gluten and fish oil (total P content 7.5 g/kg dry matter feed)¹⁰ was either not supplemented (control) or supplemented with OPTIPHOS® L 8000 to provide phytase at 250, 500, 750, 50,000 or 100,000 (100× the maximum recommended dose) OTU/kg feed. The phytase activity was confirmed by analysis. A positive control (total P content 10.8 g/kg dry matter feed)¹¹ was also considered. Pelleted feed was hand distributed avoiding feed wastage for 91 days. Temperature in the tanks was $14.2 \pm 0.3^\circ\text{C}$ and dissolved oxygen levels were kept above 6.5 mg/L. Survival and health status of fish were monitored. Feed intake was recorded and fish were weighed individually on day 0 and then, were group-weighted on days 33, 66 and 91 of the experiment. From the data, specific growth rate (% body weight per day), feed to gain ratio, relative daily feed intake (% of body weight per day) and protein efficiency ratio were calculated. At the end of the study (day 91), eight fish per tank (32 fish per treatment) were anaesthetised, individually weighed, length measured, blood sampled to determine biochemistry parameters,¹² externally examined and then dissected for gross pathology examination of the following organs: skin, gills, head kidney, viscera and liver. The liver of these fish was weighed. An analysis of variance (ANOVA) was done with the data obtained and group means were compared with the Student–Newman–Keuls test. Differences were considered significant at a level of at least $p < 0.05$.

No fish died during the study and no signs of fish distress were observed throughout the experiment. Final body weight of the fish was 130, 132, 143, 147, 155, 157 and 143 g for the control, 250, 500, 750, 50,000 and 100,000 OTU/kg feed and positive control, respectively, and the corresponding values for the feed to gain ratio were 0.96, 0.91, 0.90, 0.89, 0.88, 0.88 and 0.90 (for details, see Table 1 in Section 3.3). The body weight of the fish was higher for groups receiving 500 OTU/kg feed and higher and the feed to gain ratio was lower with the phytase at any dose (from 250 OTU/kg feed) compared to the control diet. No differences were found in the biochemical parameters measured and no findings were reported from the macroscopic analysis of the animals.

The supplementation of the experimental diets with OPTIPHOS® L 8000 at up to 100 times the maximum recommended dose did not have any negative effect on the performance or health of juvenile rainbow trout. Therefore, the additive is considered safe for the rainbow trout at maximum dose (1,000 OTU/kg feed). Based on the wide margin of safety, the FEEDAP Panel extrapolates this conclusion to all finfish at the same maximum dose (1,000 OTU/kg feed).

3.3. Efficacy for fish

Three trials performed in two different trial sites were submitted. Two were done with rainbow trout (*O. mykiss*) and one with Atlantic salmon (*Salmo salar*).

The first efficacy study was the tolerance study,⁹ already described in Section 3.2.

The second efficacy study in rainbow trout was performed at the same trial site and followed the same experimental design as the first study. A total of 700 juvenile rainbow trout (*O. mykiss*) were reared in standard conditions for approximately 3 months. Afterwards and with an initial body weight of 11.7 ± 0.5 g, fish were distributed in groups of 35 to 20 tanks and allocated to five dietary

⁹ Technical dossier/Supplementary information December 2016/Reference 2.

¹⁰ Calcium content of 5.7 g/kg dry matter feed.

¹¹ Calcium content of 8.4 g/kg dry matter feed.

¹² Including: Glucose, total protein, phosphorus and alkaline phosphatase activity.

treatments (four replicate tanks per treatment).¹³ A basal diet based on fish and krill meal, soy and pea protein concentrate, guar meal, wheat and corn gluten and fish oil (total P content 7.5 g/kg dry matter feed)¹⁰ was either not supplemented (control) or supplemented with OPTIPHOS® L 8000 to provide phytase at 250, 500 or 750 OTU/kg feed. The phytase activity was confirmed by analysis. A positive control (total P content 11.4 g/kg dry matter feed)¹⁴ was also considered. Pelleted feed was hand distributed avoiding feed wastage for 90 days. Temperature in the tanks was $13.9 \pm 0.2^\circ\text{C}$ and dissolved oxygen levels were kept above 7.6 mg/L.

The third trial was performed with 984 Atlantic salmon (*S. salar*) with an initial body weight of 163 ± 14 g. Fish were distributed in groups of 82 fish to 12 cages and allocated to four dietary treatments (three replicate tanks per treatment).¹⁵ A basal diet based on fish and krill meal, soy and pea protein concentrate, wheat and corn gluten, rapeseed meal and fish oil (total P content 8.0 g/kg dry matter feed)¹⁶ was either not supplemented (control) or supplemented with OPTIPHOS® L 8000 to provide phytase at 500 or 750 OTU/kg feed. The phytase activity was confirmed by analysis. A positive control (total P content 11.7 g/kg dry matter feed)¹⁷ was also considered. Pelleted feed was hand distributed avoiding feed wastage for 61 days. Average temperature was $12.8 \pm 1.3^\circ\text{C}$, salinity averaged 32.4 ± 0.5 g/kg and dissolved oxygen levels were kept above 5.9 mg/L.

In all studies, survival of the animals was monitored, feed distributed and body weight were measured throughout the study and specific growth rate, feed to gain ratio, relative daily feed intake and protein efficiency ratio were calculated. In the three studies, digestibility and retention of phosphorus were also measured. At the beginning and at end of the experiments, two pools of six fish at the beginning or six fish per replicate at the end were analysed for whole-body phosphorus content and for the fish bone (vertebrae) ash content. Phosphorus retention was calculated considering the phosphorus intake and the phosphorus content of the body. The fish bones from two pools of three fish per replicate tank were collected and analysed for ash content. After the end of the growth performance experimental periods, a digestibility experiment followed. The fish were fed the same diets as previously, but containing yttrium oxide as an external marker, for 14 or 7 days for the trials done in rainbow trout (trials 1 and 2) or the one done in Atlantic salmon (trial 3), respectively. Faecal samples from 15 (trial 1) or 12 fish (trials 2 and 3) were collected by stripping the ventral abdominal area for 6 or 4 h (trout or salmon, respectively) following the last meal. Feed and excreta were analysed to determine for total and phytate phosphorus in order to calculate the digestibility.

In all the studies, an ANOVA was performed on the data obtained and mean values were compared with the Student–Newman–Keuls test. The experimental unit was the tank. Differences were considered significant at a level of at least $p < 0.05$.

Results are shown in Table 1. No mortality was registered in the studies. In the three trials, the performance and the phosphorus retention (total in trial 2 and 3, and digestibility plus partial retention in trial 1) were improved by the additive at the lowest tested dose (in rainbow trout at 250 OTU/kg feed and in Atlantic salmon at 500 OTU/kg feed). Therefore, the FEEDAP Panel concludes that the additive has a potential to be efficacious in rainbow trout and salmon at 500 OTU/kg, in diets where the substrate for the phytase is present. Considering that the mode of action of the phytase is well-known and that fish have similar digestive physiology, the FEEDAP Panel considers that this conclusion can be extrapolated to all finfish species.

¹³ Technical dossier/Supplementary information December 2016/Reference 1.

¹⁴ Calcium content of 8.3 g/kg dry matter feed.

¹⁵ Technical dossier/Supplementary information December 2016/Reference 3.

¹⁶ Calcium content of 7.6 g/kg dry matter feed.

¹⁷ Calcium content of 10.9 g/kg dry matter feed.

Table 1: Effects of OPTIPHOS® on the final body weight and feed to gain ratio and P digestibility and retention parameters, whole body P content and bone ash content

Trial	Groups (OTU/kg feed)	Final body weight (g)	Feed to gain ratio	Digestibility of phosphorus (%)		Phosphorus retention (%)	Whole body P content (%) ²	Bone Ash (%) ²
				Total	Phytate			
1	Control	130.1 ^c	0.96 ^a	31.9 ^d	28.3 ^b	42.9 ^b	0.98 ^b	19.9 ^c
	250	131.9 ^c	0.91 ^b	42.1 ^c	34.7 ^b	44.8 ^b	1.00 ^b	23.2 ^b
	500	142.6 ^b	0.90 ^b	67.9 ^b	42.9 ^a	60.8 ^a	1.25 ^a	28.4 ^a
	750	146.8 ^b	0.89 ^b	72.3 ^a	50.6 ^a	60.8 ^a	1.26 ^a	28.4 ^a
	50,000	155.4 ^a	0.88 ^b	NA ¹	NA	61.0 ^a	1.26 ^a	29.1 ^a
	100,000	156.7 ^a	0.88 ^b	NA	NA	62.5 ^a	1.26 ^a	28.0 ^a
	Positive control	142.9 ^b	0.90 ^b	65.8 ^b	29.1 ^b	38.6 ^c	1.21 ^a	29.7 ^a
2	Control	58.0 ^c	0.96	32.4 ^d	31.1 ^c	42.9 ^c	1.10 ^b	20.6 ^b
	250	62.5 ^b	0.93	43.5 ^c	39.4 ^{b,c}	47.1 ^b	1.12 ^b	26.0 ^a
	500	66.1 ^a	0.92	66.2 ^b	44.4 ^{a,b}	56.4 ^a	1.29 ^a	26.4 ^a
	750	68.1 ^a	0.93	79.8 ^a	57.4 ^a	58.4 ^a	1.33 ^a	27.3 ^a
	Positive control	69.2 ^a	0.93	60.6 ^b	30.5 ^c	39.8 ^d	1.33 ^a	25.7 ^a
3	Control	379 ^a	1.03 ^a	39.3 ^b	35.4 ^b	24.4 ^b	0.30	4.9 ^c
	500	386 ^{ab}	0.97 ^b	68.1 ^a	43.2 ^a	31.3 ^a	0.32	6.8 ^b
	750	398 ^b	0.93 ^b	69.5 ^a	41.1 ^a	36.6 ^a	0.33	8.1 ^a
	Positive control	397 ^b	0.94 ^b	64.8 ^a	34.4 ^b	20.5 ^c	0.31	8.2 ^a

¹: NA: not analysed.²: Values expressed in dry matter basis in trials 1 and 2 and fresh matter basis in trial 3.^{a,b,c,d}: Values within a trial and within a column with a different superscript are significantly different ($p < 0.05$).

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 Regulation¹⁸ and Good Manufacturing Practice.

4. Conclusions

The new use of the additive would not change the previous conclusions regarding the safety for the consumer, user and environment. The FEEDAP Panel concluded that there are no concerns for consumer safety and no risks for the environment are expected from the use of the OPTIPHOS® as an additive in feed for all finfish. The additive is not a skin/eye irritant or a skin sensitiser, but has the potential to be a respiratory sensitiser.

The additive is safe for all finfish at 1,000 OTU/kg feed and it has the potential to improve the performance and increase phosphorus retention in all finfish at 500 OTU/kg feed.

Documentation provided to EFSA

- 1) OPTIPHOS® for all finfish. April 2016. Submitted by Huvepharma EOOD.
- 2) OPTIPHOS® for all finfish. Supplementary information. December 2016. Submitted by Huvepharma EOOD.
- 3) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for OPTIPHOS®.

References

EFSA (European Food Safety Authority), 2008. Technical Guidance: extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition. EFSA Journal 2008;6(9):803, 5 pp. doi:10.2903/j.efsa.2008.803

¹⁸ 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.

- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011a. Technical guidance: tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. doi:10.2903/j.efsa.2011.2175
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011b. Scientific Opinion on the safety and efficacy of Optiphos® (6-phytase) as a feed additive for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, laying hens, other birds for fattening and laying, weaned piglets, pigs for fattening and sows. EFSA Journal 2011;9(11):2414, 29 pp. doi:10.2903/j.efsa.2011.2414
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. doi:10.2903/j.efsa.2012.2536
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance for establishing the safety of additives for the consumer. EFSA Journal 2012;10(1):2537, 12 pp. doi:10.2903/j.efsa.2012.2537
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. doi:10.2903/j.efsa.2012.2539
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific Opinion on the modification of the terms of the authorisation of OPTIPHOS® (6-phytase) as a feed additive for pigs for fattening. EFSA Journal 2015;13(7):4200, 8 pp. doi:10.2903/j.efsa.2015.4200

Abbreviations

ANOVA	analysis of variance
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for OPTIPHOS®

In the current application, authorisation is sought under article 4(1) for OPTIPHOS® under the category 'zootechnical additives', functional groups 4(a) 'digestibility enhancers' according to Annex I of Regulation (EC) No 1831/2003. This feed additive is currently authorised for avian and porcine species. In the frame of this application, authorisation is sought for its use in Salmonidae and other finfish. The active agent is 6-phytase, produced by *Komagataella pastoris*.

The enzymatic activity of the active agents is expressed in 'OTU' units, where 'one OTU is the amount of enzyme that catalyses the release of one micromole of inorganic phosphate per minute from 5.1 mM sodium phytate in pH 5.5 citrate buffer at 37°C, measured as the blue phosphorus-molybdate complex colour at 820 nm'.

The *feed additive* is intended to be marketed as two granulate formulations (*G* 4000 and *CT* 4000) and one liquid formulation (*L* 8000) with minimum activities of 4,000 OTU/g, and of 8,000 OTU/g for the granulated and liquid formulations, respectively. The Applicant proposed a minimum activity for 6-phytase in complete *feedingstuffs* of 250 OTU/kg for Salmonidae and other finfish.

For the quantification of phytase activity in *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted the same single-laboratory validated and further verified colorimetric method evaluated by the EURL in the frame of the dossier FAD-2010-0008, based on the quantification of the inorganic phosphate released by the enzyme from the sodium phytate. Supplementary experimental results were provided for feedingstuffs for rainbow trout. Based on the satisfactory performance characteristics available, the EURL recommends for official control the colorimetric method mentioned above for the quantification of *phytase* activity in the *feed additive*, *premixtures* and *feedingstuffs*.

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