Safety and efficacy of Natuphos® E (6-phytase) as a feed additive for avian and porcine species

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Abstract

Natuphos® E is a feed additive that contains a 6-phytase available in powder, granulated and liquid forms which is intended to be used as a feed additive for avian and porcine species. The production strain of the phytase present in the product is a genetically modified strain of *Aspergillus niger*. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the genetic modification of the production strain does not give rise to safety concerns. The production strain and its DNA were not detected in the concentrate used to formulate the products. 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 Panel also concluded that the use of the product as a feed additive does not give rise to concerns for consumers. Evidence was provided showing that the additive is not toxic by inhalation or irritant for skin or eyes, however, it should be regarded as a dermal sensitiser and a potential respiratory sensitiser. The use of the additive as a feed additive poses no risks to the environment. Based on the efficacy studies provided, the Panel concluded that the additive has the potential to be efficacious in chickens for fattening, turkeys for fattening, piglets, pigs for fattening and sows. These conclusions were extended to chickens reared for laying, turkeys reared for breeding and extrapolated to minor poultry species and other avian species for fattening and to the point of lay and to minor porcine species. The Panel considered that there was insufficient information to conclude on the efficacy in laying hens.

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Keywords: safety, efficacy, zootchnical additives, 6-phytase, poultry and pigs

Requestor: European Commission

Question numbers: EFSA-Q-2015-00074 and EFSA-Q-2015-00732

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**Amendment:** This scientific output replaces the earlier version published on 14 November 2017. The updated version of this EFSA output is published after the withdrawal of the confidentiality claim by the applicant.

**Suggested citation:** EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Dierick NA, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M and Cocconcelli PS, 2017. Scientific opinion on the safety and efficacy of Natuphos® E (6-phytase) as a feed additive for avian and porcine species. EFSA Journal 2017;15(11):5024, 35 pp. https://doi.org/10.2903/j.efsa.2017.5024

**ISSN:** 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.
Summary

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 Natuphos® E (6-phytase) as a feed additive for avian and porcine species.

The additive Natuphos® E presents 6-phytase (phytase; Enzyme Commission Number 3.1.3.26) and it is intended to be used as a feed additive for all avian and pig species as a zootechnical additive, functional group of digestibility enhancers and substances which favorably affect the environment. The phytase present in the additive is produced by a genetically modified strain of Aspergillus niger. The FEEDAP Panel concluded that the genetic modification of the production strain does not give rise to safety concerns. The production strain and its DNA were not detected in the concentrate used to formulate the additive.

The results of the tolerance trials showed that chickens and turkeys for fattening tolerated 400-fold the minimum recommended dose, laying hens 250-fold, weaned piglets 500-fold and sows 25-fold. Therefore, the FEEDAP Panel concluded that the additive is safe at the respective minimum recommended levels, namely chickens for fattening 125 FTU/kg feed, turkeys for fattening 250 FTU/kg feed, laying hens 200 FTU/kg feed and piglets and sows at 100 FTU/kg feed, with a wide margin of safety. The conclusions were extended to chickens reared for laying, turkeys reared for breeding and pigs for fattening and extrapolated to other avian species and to porcine species.

The results obtained with the enzyme concentrates in the genotoxicity studies and in the subchronic oral toxicity study did not indicate any reason for concern for consumer safety arising from the use of the product as a feed additive. Therefore, the Panel considered that the additive is safe for the consumers of food products derived from animals fed with the additive.

The solid concentrate was not toxic by inhalation, not irritant to skin or eyes but showed a potential to be a dermal sensitiser, which has been demonstrated in exposed workers. No data were submitted for the liquid concentrate. Taking into account the composition of the final formulations, the Panel considered that the conclusions reached for the solid concentrate apply to the final formulations of the additive. Owing to the proteinaceous nature of the active substance, the FEEDAP Panel concluded that the additive has also to be considered a potential respiratory sensitiser.

The Panel concluded that the final product does not pose any environmental safety concern associated with the genetic modification. The active substance of the additive is a protein, and as such will be degraded/inactivated during passage through the digestive tract of animals or in the environment. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

The Panel assessed efficacy studies done in chickens for fattening, turkeys for fattening, laying hens, piglets, pigs for fattening and sows. Based on the results obtained, the Panel concluded that the additive has the potential to be efficacious in improving the performance and/or the phosphorus utilisation in chickens for fattening at 750 FTU/kg feed, in turkeys for fattening and weaned piglets at 125 FTU/kg feed and in pigs for fattening and sows at 100 FTU/kg feed. The conclusions on the chickens and turkeys for fattening were extended to chickens and turkeys reared for laying/breeding at the corresponding dose. Taking account of the mode of action of phytases, the conclusions drawn in turkeys for fattening were extrapolated to all minor poultry species and other avian species for fattening or up to the point of lay. Similarly, the conclusions drawn in pigs for fattening and sows were extrapolated to minor porcine species for growing and reproduction, respectively. The Panel concluded that there was insufficient data to conclude on the efficacy of the additive in laying hens and therefore could not conclude on minor poultry and other avian species for laying.
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1. **Introduction**

1.1. **Background and Terms of Reference**

Regulation (EC) No 1831/2003\(^1\) establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received two requests from BASF SE\(^2\) for authorisation of the product Natuphos\(^®\) E (6-phytase), when used as a feed additive for all avian species and all pigs (category: zootechnical additives; functional group: digestibility enhancers and substances which favorably affect the environment).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossiers in support of the applications. The particulars and documents in support of the application were considered valid by EFSA as of 25 March 2015 and 7 January 2016, respectively.

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 Natuphos\(^®\) E (6-phytase), when used under the proposed conditions of use (see Section 3.1.5).

2. **Data and methodologies**

2.1. **Data**

The present assessment is based on data submitted by the applicant in the form of technical dossiers\(^3\) in support of the authorisation request for the use of Natuphos\(^®\) E as a feed additive. The technical dossiers were prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008\(^4\) and the applicable EFSA guidance documents.

EFSA has verified the European Union Reference Laboratory (EURL) reports as they relate to the methods used for the control of the Natuphos\(^®\) E in animal feed. The Executive Summaries of the EURL reports can be found in Appendix A.\(^5\)

2.2. **Methodologies**

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Natuphos\(^®\) E 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, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c), Technical Guidance: Microbial Studies (EFSA, 2008b), Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008c), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012d) and Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011).

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\(^1\) 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.

\(^2\) BASF SE, ENS/LR – F31, Chemiestrasse 22, 68623 Lampertheim, Germany.

\(^3\) FEED dossier reference: FAD-2014-0044 and FAD-2015-0040.

\(^4\) 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 reports are available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/fnirep-fad-2014-0044-natuphose.pdf and https://ec.europa.eu/jrc/sites/jrcsh/files/fnirep-fad-2015-0040-natuphos_e.pdf
3. **Assessment**

The additive Natuphos® E presents 6-phytase (phytase; Enzyme Commission Number 3.1.3.26) and it is intended to be used as a feed additive for all avian and pig species as a zootechnical additive, functional group of digestibility enhancers and substances which favorably affect the environment.

3.1. **Characterisation**

3.1.1. **Characterisation of the active substance**

The phytase present in Natuphos® E is produced by a genetically modified strain of *Aspergillus niger*, which is deposited at the German Collection of Microorganisms and Cell Cultures with the accession number DSM 25770.6

3.1.1.1. **Information relating to the genetically modified microorganism**

**Characteristics of the recipient or parental microorganism**

The production organism is the filamentous fungus *Aspergillus niger* and was identified as *A. niger* by sequence analysis of the ITS regions.7 The parental *A. niger* strain GAM-53 was obtained from the wild-type *A. niger* NRRL 3122 by classical mutagenesis. The recipient strain *A. niger* ISO-502 was derived from GAM-53 by genetic modification, including the replacement of the seven *glaA* loci with truncated non-functional *ΔglaA* sequences. These seven loci, into which various expression units can be integrated, differ in length and are marked with unique restriction enzyme cleavage sites (Selten et al., 1998). Genetic modification was also used to inactivate the gene coding for the major extracellular protease (*pepA*). The recipient strain is free from antibiotic resistance genes or other marker genes. This strain has been previously assessed by the EFSA FEEDAP and GMO Panels (2008).

**Characteristics of the donor organisms**

The synthetic gene encoding 6-phytase (called HF586) was constructed based on the 6-phytase genes of the enterobacteria *Hafnia* sp., *Yersinia mollaretii* and *Buttiauxella gaviniae*. The gene was codon-optimised and further modified to improve the stability at high temperature, acidic pH profile and high specific activity of the encoded protein.8 Blast analysis indicated that the active site of HF586 is unchanged compared to that in the native phytase in *Hafnia* sp. The *P* glaA promoter and the *phyA* signal sequence (SS) for protein secretion are derived from *A. niger*. The *P* gpd promoter and the *amdS* sequence are derived from *Aspergillus nidulans*. The *amdS* gene encodes acetamidase, which enables the use of acetamide as a sole nitrogen and carbon source, and was used as a selectable marker.

**Description of the genetic modification process**

A DNA fragment consisting of the HF586 gene, the *P* glaA promoter and the *phyA* signal sequence, was constructed by fusion polymerase chain reaction (PCR). This fragment was introduced into the plasmid pGBTOP.9 Plasmid pGBTOP is based on the commercially available *Escherichia coli* plasmid pTZ18R, and carries the *P* glaA, flanked at both ends by a ca. 2-kb fragment of the 3’ flank of the *A. niger* GAM-53 *glaA* terminator sequence. The plasmid pGBTOP also carries the *bla* gene from pTZ18R. The *P* glaA-SS-HF586 fragment was introduced in such a way that it was located between the two *glaA* flanking sequences. The resulting plasmid was called pGBTOP-HF586.10 From pGBTOP-HF586, an expression cassette consisting of the *P* glaA-SS-HF586 sequence including the two *glaA* flanking sequences (the *bla* gene was not included) was isolated by digesting with restriction endonuclease and purified by agarose gel electrophoresis.

In parallel, the *amdS* gene was cloned into pGBTOP, resulting in the plasmid pGBAAS-1.11 From pGBAAS-1, an expression cassette consisting of the *P* gpd-*amdS* sequence flanked by the *glaA* sequences (the *bla* gene not included) was isolated by digesting with restriction endonuclease and purified by agarose gel electrophoresis.

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6 Technical dossier FAD-2014-0044/Section II/Annex II 52.
7 Technical dossier FAD-2014-0044/Section II/Annex II 53.
8 Technical dossier FAD-2014-0044/Section II/Annex II 41.
9 Technical dossier FAD-2014-0044/Section II/Annex II 44.
10 Technical dossier FAD-2014-0044/Section II/Annex II 45.
11 Technical dossier FAD-2014-0044/Section II/Annex II 46.
The two expression cassettes were co-introduced into the recipient strain ISO-502 by protoplast incubation in the presence of polyethylene glycol, and integrated into one or more glaA loci by homologous recombination. Transformants were selected by their ability to grow on acetamide as a sole carbon/nitrogen source. A transformant with the highest phytase production was selected. A strain lacking the amdS gene due to natural recombination was selected by its ability to grow in the presence of fluoro-acetamide (which is degraded into the toxic fluoroacetic acid by acetamidase). This strain was subjected to another round of co-transformation, selection and elimination of the marker, to finally select the production strain with the highest phytase production, which was designated LU17257.

Southern analysis showed that the production strain LU17257 carries 13 tandem repeats of the P_glaA-SS-HF586 expression cassette located in two of the seven glaA loci. Due to natural recombination events among the glaA loci, some of the tandem repeats are amplified or lost during a 168 h fermentation cycle, but they are not incorporated into other parts of the genome. Southern analysis also showed the absence from the production strain of the amdS gene and of sequences derived from pTZ18R, including the bla gene.

3.1.2. Manufacturing process

The additive is available in one powder formulation (Natuphos® E 5000), two granulated formulations (Natuphos E 5000 G and 10000 G) and two liquid formulations (Natuphos® E 5000 L and Natuphos® E 10000 L).

The phytase is obtained by submerged aerobic fermentation of the production strain followed by a recovery and downstream processing.

The resulting product (liquid concentrate) is mixed with glycerol to prepare the liquid formulations or mixed with magnesium sulfate and then spray-dried to prepare the powder formulation. The spray-dried product is referred as Natuphos® E SD powder (solid concentrate) and it is mixed with bran to prepare Natuphos® E 5000. The granular formulations are prepared using the liquid concentrate which is mixed with polyvinylalcohol, gum arabic, coating agent and starch, and dried to prepare Natuphos® E 5000 G and 10000 G. No antimicrobials are used in the manufacturing of the product.

3.1.3. Characterisation of the additive

The powder formulation, Natuphos® E 5000, contains the phytase concentrate and magnesium sulfate (~ 2%), wheat bran (97%), and vegetable oil (~ 1%, soya bean). This formulation ensures a minimum activity of 5,000 FTU/g. The study of the batch-to-batch variation in five batches showed a mean value of 5,372 FTU/g ranging from 5,210 to 5,690 FTU/g (coefficient of variation (CV) of 3.5%). This formulation has a bulk density of 348 kg/m³. Particle size distribution was studied in three batches of this formulation and showed a mean particle size of 368-374 μm. Particles below 100, 50 and 10 μm amount to 5%, 2.6% and 0.1%, respectively. Dusting potential of three batches, measured with a Heubach Dustmeter Type II, ranged from 0.010 to 0.020 g/m³.

The granular formulations, Natuphos® E 5000 G and 10000 G, contain the phytase concentrate (from 1.5% to 2.7%), starch (82%), polyvinylalcohol (1.4%), gum arabic (3%), wax-based coating agent (5.0%) and water (up to 100%). Natuphos® E 5000 G and 10000 G ensure a minimum activity of 5,000 and 10,000 FTU/g, respectively. The study of the batch-to-batch variation in five batches of Natuphos® E 5000 G showed a mean value of 7,770 FTU/g ranging from 5,710 to 8,630 FTU/g (CV of 16%), and in five batches of Natuphos® E 10000 G showed a mean value of 12,010 FTU/g ranging from 11,340 to 13,180 FTU/g (CV of 6.4%). The bulk density of these formulations is similar to 650 kg/m³. Particle size was studied in three batches of these formulations and showed a mean particle size of 600 μm, particles below 850 μm > 90%, below 710 μm > 80%, below 600 μm > 50%.

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12 Technical dossier FAD-2014-0044/Section II/Annex II 51.
13 Technical dossier FAD-2014-0044/Section II/Annex II 55.
14 Technical dossier FAD-2014-0044/Section II/Annex II 54.
15 Technical dossier FAD-2015-0040/Section II/Annex II.4.
16 Technical dossier FAD-2014-0044/Section II/Annex II 55.
17 Technical dossier FAD-2014-0044/Section II/Annex II 64.
18 Technical dossier FAD-2015-0040/Section II/Annex II.14 and II.15.
and below 63 \( \mu \text{m} \) 0.01–0.08%.\(^{22}\) Dusting potential of three batches, measured with a Heubach Dustmeter Type II, was 0.010 g/m\(^3\) for Natuphos\(^{\circledR}\) E 5000 G and from 0.01 to 0.03 g/m\(^3\) for Natuphos\(^{\circledR}\) E 10000 G.\(^{23}\)

The liquid formulations, Natuphos\(^{\circledR}\) E 5000 L and Natuphos\(^{\circledR}\) E 10000 L, contain the phytase concentrate (from 1.2% to 2.5%), glycerol (50%), sodium benzoate (0.15%) and water. Natuphos\(^{\circledR}\) E 5000 L and Natuphos\(^{\circledR}\) E 10000 L, ensure a minimum of 5,000 and 10,000 FTU/g product, respectively. The study of the batch-to-batch variation in five batches of Natuphos\(^{\circledR}\) E 5000 L showed a mean value of 6,524 FTU/g ranging from 6,390 to 6,610 (CV of 1.3%) and in three batches of Natuphos\(^{\circledR}\) 10,000 L showed a mean value of 11,250 FTU/g ranging from 11,230 to 11,290 FTU/g.\(^{24}\)

The formulations have a density of 1.2 g/cm\(^3\) and a pH of 3.3 and below 6.3.

The liquid intermediate was analysed for chemical and microbiological contamination.\(^{25}\) The analyses of chemical contamination included arsenic (\(< 0.04 \text{ mg/kg}\)), cadmium (\(< 0.01 \text{ mg/kg}\)), lead (\(< 0.1 \text{ mg/kg}\)), mercury (\(< 0.001 \text{ mg/kg}\)), fluorine (\(< 10 \text{ mg/kg}\)), polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (WHO-TE \( \leq 0.030 \text{ ng/kg}\)), dioxin-like polychlorinated biphenyls (PCBs) (WHO TE \( \leq 0.005 \text{ ng/kg}\)), non-dioxin like PCBs (\( \leq 3.12 \mu\text{g/kg}\)). The following mycotoxins were analysed in the solid and liquid intermediate aflatoxin B1 (\(< 0.3 \mu\text{g/kg}\)), zearalenone (\(< 1 \mu\text{g/kg}\)), ochratoxin A (\(< 5 \mu\text{g/kg}\)), fumonisin B1 + B2 (\(< 20 \mu\text{g/kg}\)) and deoxynivalenol (\(< 10 \mu\text{g/kg}\)).

The species to which the production strain belongs is known to be capable of producing secondary metabolites of toxicological relevance other than mycotoxins (e.g. malformins, naphthopyrones, nigerazines, nigragillin and oxalic acid). Although requested, the applicant failed to provide data on this aspect.

Microbiological analysis included Salmonella spp. (absence in 25 mL), E. coli (absence in 25 mL), enterobacteria ceae (\(< 1 \text{ colony forming units (CFU)/g}\)), coliforms (\(< 1 \text{ CFU/g}\)), total viable counts (\(< 50 \text{ CFU/g}\)), yeasts and moulds (\(< 50 \text{ CFU/g}\)).

Three batches of Natuphos\(^{\circledR}\) E 5000,\(^{26}\) Natuphos\(^{\circledR}\) E 5000 G\(^{27}\) and Natuphos\(^{\circledR}\) E 5000 L\(^{28}\) were analysed for chemical and microbiological contamination.\(^{29}\) The analyses of chemical contamination included arsenic (\(\leq 0.25 \text{ mg/kg}\)), cadmium (\(\leq 0.08 \text{ mg/kg}\)), lead (\(\leq 0.33 \text{ mg/kg}\)), mercury (\(\leq 0.001 \text{ mg/kg}\)), fluorine (\(\leq 10 \text{ mg/kg}\)), polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (WHO-TE \(\leq 0.141 \text{ ng/kg}\)), dioxin-like PCBs (WHO TE \(\leq 0.026 \text{ ng/kg}\)) and non-dioxin like PCBs (\(\leq 2.84 \mu\text{g/kg}\)). The following mycotoxins were analysed: aflatoxin B1 (\(\leq 0.3 \mu\text{g/kg}\)), zearalenone (\(\leq 1 \mu\text{g/kg}\)), ochratoxin A (\(\leq 5 \mu\text{g/kg}\)), fumonisin B1 + B2 (\(\leq 20 \mu\text{g/kg}\)) and deoxynivalenol (\(\leq 138 \mu\text{g/kg}\)).

Methanol was analysed in the granular form and found to be \(< 0.001 \text{ mg/kg}\). Microbiological analysis included Salmonella spp. (absence in 25 g or mL), E. coli (absence in 25 g or mL), enterobacteria (\(\leq 1.1 \times 10^5 \text{ CFU/g in Natuphos\(^{\circledR}\) E 5000; } < 1 \text{ CFU/g or mL in the other formulations}\)), coliforms (\(\leq 2.1 \times 10^6 \text{ CFU/g in Natuphos\(^{\circledR}\) E 5000; } < 1 \text{ CFU/g or mL in the other formulations}\)), total viable counts (\(\leq 1.55 \times 10^6 \text{ CFU/g in Natuphos\(^{\circledR}\) E 5000; } 2 \times 10^3 \text{ CFU/g in Natuphos\(^{\circledR}\) E 5000 G and } < 1 \text{ CFU/g or mL in Natuphos\(^{\circledR}\) E 5000 L, yeasts and moulds in the liquid formulation } (\leq 50 \text{ CFU/g})\), and in the solid formulations Pseudomonas aeruginosa (absence in 25 g, except in one batch of 5000 G) and Staphylococcus aureus (\(\leq 25 \text{ CFU/g}\)). The Panel notes the high counts found in total viable bacteria, coliforms and enterobacteria found in Natuphos\(^{\circledR}\) E 5000 which may be derived from the ingredients (e.g. bran) used to formulate the product.\(^{30}\) Antimicrobial activity was tested in the three formulations and found to be absent in all batches tested with the exception of two batches of Natuphos\(^{\circledR}\) E 5000, which showed inhibition of one of the strains tested (Micrococcus luteus). However, the samples were also tested for the presence of 27 substances with antimicrobial activity and all of them were below the limits of detection.

The production strain was not detected in a test volume of 1 mL of three independent samples of the concentrated product before formulation, tested in triplicate by culturing on agar plates under selective conditions for the production strain for 10 days at 30°C.\(^{31}\) No recombinant DNA was detected in three 200-mg samples of the concentrated product before formulation, obtained from three

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\(^{22}\) Technical dossier FAD-2015-0040/Section II/Annex II.16 and II.17.
\(^{23}\) Technical dossier FAD-2015-0040/Section II/Annex II.18.
\(^{24}\) Technical dossier FAD-2014-0044/Section II/Annex II.14 and Supplementary information February 2017/Annex 4.
\(^{25}\) Technical dossier FAD-2014-0044/Section II/Annex II.22 and Supplementary information February 2017/Annex 64.
\(^{26}\) Technical dossier FAD-2014-0044/Section II/Annex II.10 to 14.
\(^{27}\) Technical dossier FAD-2015-0040/Section II/Annex II.8, II.9, II.11, II.13 and II.71.
\(^{28}\) Technical dossier FAD-2014-0044/Section II/Annex II.15–II.20.
\(^{29}\) The values given within brackets show the highest value obtained in the batches studied when values were essentially in the same range for the three formulations.
\(^{30}\) Technical dossier FAD2014-0044/Supplementary information February 2017/Annex 1.
\(^{31}\) Technical dossier FAD-2014-0044/Section II/Annex II.23.
independent production batches and tested in triplicate. Analysis was performed by PCR, amplifying a 507-bp fragment spanning the 5’ flanking sequence and the initial part of the HF586 gene, and a 1,4-kb fragment corresponding to the almost complete HF586 gene.32

3.1.4. Stability and homogeneity

No shelf-life/stability or homogeneity studies were provided for the solid formulation Natuphos® E 5000. The applicant submitted information for the spray-dried product, granulated and liquid. The materials added to the spray-dried product to prepare Natuphos® E 5000, basically bran, are not expected to modify the stability/homogeneity properties of the phytase, therefore such data can be used to study the stability and homogeneity of Natuphos® E 5000. In the text below, the results of those studies will be referred to belong to Natuphos® E 5000 formulation.

3.1.4.1. Shelf-life

The shelf-life of Natuphos® E 5000 was studied in samples from three batches stored for up to 24 months at 20°C or 30°C in closed paper bags.33 In samples stored at 20°C, recoveries of the initial activity after 6 months were > 95%, after 18 months were > 85% and after 24 months > 80%. Samples stored at 30°C showed recoveries > 70% after 3 months and below 50% after 6 months.

The shelf-life of Natuphos® E 5000 G and 10000 G was studied in three batches of each formulation stored for up to 15 months at 20°C or 30°C in closed plastic bags.34 In samples stored at 20°C, recoveries after 6 and 15 months were > 90%, regardless of the formulation. In samples stored at 30°C, recoveries for Natuphos® E 5000 G and 10000 G were, respectively, > 84% and 85% after 2 months, > 75% and 68% after 6 months, and > 49% and 41% after 15 months.

The shelf-life of the liquid formulations Natuphos® E 5000 L and Natuphos® E 10000 L was studied in samples from three batches of each formulation that were kept at 6, 20 or 30°C in polyethylene bottles.35 In samples kept at 6°C or at 20°C, mean recoveries after 18 months were higher than 98% of the initial activity. For samples kept at 30°C, recoveries after 15 months were higher than 80% of the initial activity.

3.1.4.2. Stability and homogeneity in feedingstuffs

Three batches Natuphos® E 5000 powder,36 Natuphos® E 5000 G and 10000 G37 were added to a mineral vitamin premixture (without choline chloride) at a dose of 500,000 FTU/kg and samples were stored in closed paper bags at 20°C or 30°C for up to 6 months. In samples stored at 20°C, recoveries after 6 months were > 78% for powder or > 90% for the granulated forms. In samples stored at 30°C, recoveries after 6 months were very low (20–23%) for the powder or > 72% for the granulated forms, respectively.

Three batches of Natuphos® E 5000,38 Natuphos® E 5000 G and 10000 G39 were added to a complete compound feed (premix with choline chloride) at a dose of 1,000 FTU/kg feed and then it was pelleted to have 80–85 or 90°C after die. At the temperature of 80°C, the recovery was ~ 85% for the powder, ~ 82% for Natuphos® E 5000 G and ~ 98% Natuphos® E 10000 G. At the temperature of 90°C, the recovery was ~ 50% for the powder form, ~ 70% for Natuphos® E 5000 G and ~ 88% Natuphos® E 10000 G.

Three batches of Natuphos® E 5000 powder,40 Natuphos® E 5000 G and 10000 G41 were also added to a complete compound feed (premix with choline chloride) at a dose of 1,000 FTU/kg feed and were pelleted. Samples (mash and pelleted) were stored in closed paper bags at 20°C or 30°C for up to 16 weeks. Mean recoveries in the samples stored at 20°C were > 93% and 90% for mash and pelleted samples. Mean recoveries in samples stored at 30°C were > 82% and 73% for mash and pelleted samples, respectively.

32 Technical dossier FAD-2014-0044/Section II/Annex II 24.
33 Technical dossier FAD-2014-0044/Section II/Annex II.57.
34 Technical dossier FAD-2015-0040/Section II/Annex II.57 and II.58.
35 Technical dossier FAD-2014-0044/Supplementary information February 2017/Annexes 5 and 6.
36 Technical dossier FAD-2014-0044/Section II/Annex II.59.
37 Technical dossier FAD-2015-0044/Section II/Annex II.72 and II.73.
38 Technical dossier FAD-2014-0044/Section II/Annex II.61 and Supplementary information February 2017/Annex 11.
39 Technical dossier FAD-2015-0044/Section II/Annex II.61 and II.62.
40 Technical dossier FAD-2014-0044/Section II/Annex II.60 and Supplementary information February 2017/Annex 11.
41 Technical dossier FAD-2015-0040/Section II/Annex II.59 and II.60.
The stability of Natuphos® E 5000 L and Natuphos® E 10000 L when sprayed onto the pelleted feed at a dose of 1,000 FTU/kg feed was studied in samples stored in closed paper bags at 20°C or 30°C. Mean recoveries after 3 months for the samples kept at 20°C were > 89% and 85% for 5000 L and 10000 L, respectively, and at 30°C were 57% and 61%, respectively.

The capacity to homogeneously distribute of one batch of Natuphos® E 5000, three batches of Natuphos® E 5000 G and 10000 G and one batch of Natuphos® E 5000 L was studied in complete feed. At least 10 subsamples of each batch were analysed for enzyme activity and the CV was calculated. The variation was below 10% for the solid formulations and below 3% for the liquid.

### 3.1.5. Conditions of use

The additive is to be used in feed for chickens for fattening or reared for laying at a minimum dose of 125 FTU/kg feed, turkeys for fattening or breeding, minor poultry species and ornamental birds for fattening or breeding at a minimum dose of 250 FTU/kg feed, in laying hens and minor poultry species and ornamental birds for laying at a minimum dose of 200 FTU/kg feed and in pig species at a minimum dose of 100 FTU/kg feed. No maximum dose is specified.

### 3.2. Safety

#### 3.2.1. Safety aspects of the genetic modification

The recipient strain, *A. niger* ISO-502 is considered to be safe. The data provided by the applicant confirms previous assessments (EFSA FEEDAP and GMO Panels, 2008). There are no antibiotic resistance genes in the recipient strain remaining from the genetic modification process. The traits introduced in the production strain *A. niger* DSM 25770 include 6-phytase activity. The introduced sequences raise no safety concern.

Neither the production strain nor its recombinant DNA was detected in the fermentation product. The product Natuphos® E, manufactured with the production strain *A. niger* DSM 25770, does not give rise to safety concern with regard to the genetic modification of the production strain.

#### 3.2.2. Safety for the target species

In all the studies presented in this section, the significance level was set at \( p < 0.05 \).

##### 3.2.2.1. Safety for chickens for fattening

In a combined tolerance and efficacy trial, a total of 1,680 one-day-old male chickens for fattening (Ross 308) were penned in groups of 35 birds and allocated to six dietary treatments (representing eight replicates per treatment). Two basal diets (starter and grower) based on maize and soybean meal (total phosphorus content 0.41/0.43%; calcium content 0.61/0.55%) were either not supplemented (control) or supplemented with Natuphos® E 10000 L to provide 0, 125 (1 × minimum recommended dose), 250, 500 and 50,000 (400 ×) FTU/kg diet (confirmed by analysis). The sixth treatment group was based on diet with higher phosphorus content (total phosphorus content 0.66/0.68%; calcium 0.90/0.86%). Diets were offered *ad libitum* in pelleted form for 43 days. Diets presented narasin and nicarbazin (starter) or monensin (grower), the grower diet presented titanium dioxide as an external marker. General health status of the birds was monitored throughout the study. Feed intake, body weight and mortality were recorded, and feed to gain ratio was calculated. In day 43, blood samples were obtained from two birds per pen from the 0, 500 and 50,000 FTU/kg diet as well as the positive control and analysed for haematological and biochemical parameters. An analysis of variance (ANOVA) was performed with the data (pen basis) and group means were compared with Tukey test.

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42 Technical dossier FAD-2014-0044/Supplementary information February 2017/Annexes 7 and 8.
43 Technical dossier FAD-2014-0044/Section II/Annex 63.
44 Technical dossier FAD-2014-0044/Supplementary information Annex 9.
45 Technical dossier FAD-2014-0044/Section III/Annex III.4/Supplementary information February 2017/Annex 18 and Annex 33.
46 Including: erythrocytes count, haematocrit, haemoglobin, mean corpuscular volume of red blood cells (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).
47 Including: glucose, uric acid, total proteins, calcium, phosphorus, chloride, sodium, potassium, creatinine, total bilirubin, alkaline phosphatase (ALK), aspartate aminotransferase (AST), alanine aminotransferase (ALT/GPT), gamma glutamyl transpeptidase (GGT) and lactate dehydrogenase.
Mean mortality and culling rate were below 5.0% and 1.1%, respectively, and not affected by the dietary treatments. Mean values were for daily feed intake 126 g/day for body weight 3.42 kg, and for feed to gain ratio 1.59. No adverse effects were found on the performance of the birds fed the phytase. For further details, see Section 3.3.1.2. Blood parameters measured showed no modifications by the dietary treatments with the exception of uric acid which showed lower values in the 50,000 FTU/kg and positive control diets as compared to the non-supplemented diet (326 and 355 vs 438 μmol/L). The phytase supplementation up to 400-fold the recommended dose of 125 FTU/kg feed did not adversely affect the health and performance of the birds. Therefore, the FEEDAP Panel concludes that the product is safe for chickens for fattening at the recommended dose.

3.2.2.2. Safety for turkeys for fattening

In a combined tolerance and efficacy trial, a total of 1,344 one-day-old female turkeys for fattening (BUT Big 6) were penned in groups of 24 birds and allocated to six dietary treatments (11 replicates or 9 replicates per treatment). Four basal diets (starter to grower) based on maize and soybean (total phosphorus content from 0.63% to 0.51%; calcium content from 0.92% to 0.65%) were either not supplemented (negative control) supplemented with Natuphos® E 5000 to provide 250 (1× minimum recommended dose), 500, 1,000 and 100,000 (400× recommended dose) FTU/kg feed (confirmed by analysis). A positive control diet was also considered (total phosphorus content from 0.91% to 0.70%; calcium from 1.04% to 0.71%). Diets were offered ad libitum in pelleted form for 98 days. Tolerance dose was studied until day 56. Diets presented diclazuril until day 77. General health status of the birds was monitored throughout the study. Feed intake, body weight and mortality were recorded and feed to gain ratio was calculated. An ANOVA was performed on the data (pen basis) and group means were compared with Tukey test.

Mortality and culling rate were below 5.1% and 2.3%, respectively, and showed no modifications by the dietary treatments. Mean values were for feed intake 120 g/day, for body weight 4.45 kg, and for feed to gain ratio 1.70. No significant and relevant effects were found in the turkeys receiving the phytase compared to the control. The phytase supplementation up to 400-fold the recommended dose of 250 FTU/kg feed did not adversely affect the health and performance of the birds. Therefore, the FEEDAP Panel concludes that the product is safe for turkeys for fattening at the recommended dose.

3.2.2.3. Safety for laying hens

A combined efficacy and tolerance trial was performed with 315 21-week-old Hy-Line-Brown laying hens were caged in groups of three hens and allocated to seven dietary treatments (representing 15 replicates per treatment). A basal diet based on maize and soybean meal (total phosphorus content of 0.31%; calcium content of 3.6%) was either not supplemented (negative control) or supplemented with Natuphos® E 5000 to provide 100, 200 (1× minimum recommended dose), 300, 500 and 50,000 (250× recommended dose) FTU/kg feed (confirmed by analysis). A positive control was also included (total phosphorus content of 0.49%; calcium content of 3.6%). Diets were offered ad libitum as a coarse meal for 24 weeks. Tolerance dose was studied until day 56. Health status of the animals was monitored throughout the study. Body weight and feed intake were measured every 4 weeks and feed to egg mass ratio was calculated. Number of eggs laid was monitored continually, egg-shell thickness and strength were measured every 4 weeks (30 eggs/treatment per week); Haugh units were measured every 2 weeks (10 eggs/treatment per week). An ANOVA was performed on the data (replicate basis) and group means were compared with Tukey test.

Mean feed intake was 112 g/day, mean total number of eggs produced per hen during the study period (56 days) was 52, mean egg weight was 59 g and mean feed to egg mass ratio 2.11. No statistically significant differences were found between the treatments. The phytase supplementation up to 250-fold the recommended dose of 200 FTU/kg feed did not adversely affect the health and performance of the birds. Therefore, the FEEDAP Panel concludes that the product is safe for laying hens at the recommended dose.

3.2.2.4. Safety for weaned piglets

A total of 144 weaned male and female piglets ([Duroc × Landrace] × Piétrain, 26 days old, body weight 7.3 kg) were distributed to pens in groups of three piglets each and allocated to six dietary treatments...
treatments (representing eight replicates per treatment). Basal diets (prestarter and starter) based on maize and soybean meal (total phosphorus content 0.42% and 0.40%; calcium content 0.50% and 0.48%) were either not supplemented (negative control) or supplemented with Natuphos® E 10000 L to provide 125 (1.25× minimum recommended dose), 250, 500 or 50,000 (500×) FTU/kg feed. Enzyme activities were confirmed by analysis. A positive control was also considered (total phosphorus content 0.57% and 0.56%; calcium 0.83% and 0.78%). Feeds were offered ad libitum and in pelleted form for 42 days. Health status and mortality were monitored daily. Feed intake and body weight were recorded at the beginning (only body weight), day 21 and day 42 and feed to gain ratio was calculated for the two periods. On day 42 of the experiment, blood samples were obtained from eight piglets per treatment (1 piglet per replicate; the piglet with the intermediate initial body weight was selected) from the negative control, 500, 50,000 FTU/kg and positive control for routine blood haematology and biochemistry. An ANOVA was done with the data considering the pen as the experimental unit and group means were compared by Tukey test.

No animals died during the study. Mean values were for daily feed intake 605 g/day, for final body weight 23.6 kg and for feed to gain ratio 1.57. No negative effects were observed with the phytase. For further details, check Section 3.3.4.2. The parameters analysed in blood showed significant differences between the treatments for phosphate concentration (negative control showing the lowest values compared to 500 FTU/kg and 50,000 FTU/kg) and for alkaline phosphatase that was significantly higher in the negative control compared to 500 and 50,000 FTU/kg (negative control 1,001 IU/L vs 506 and 486 IU/L, respectively). The phytase supplementation up to 500-fold the recommended dose of 100 FTU/kg feed did not adversely affect the health and performance of the piglets. Therefore, the FEEDAP Panel concludes that the product is safe for piglets for fattening at the recommended dose.

3.2.2.5. Safety for sows

A total of 36 primiparous Rattlerow Seghers sows were allocated to four dietary treatments (9 sows per treatment) and kept under study for one reproductive cycle. Sows were kept in groups of three during gestation and individually during lactation. Sows received gestation or lactation diets based on maize, barley and soybean meal (total phosphorus content 0.50% and 0.49%; calcium content 0.83% and 0.68%) that were either not supplemented (control) or supplemented with Natuphos® E 5000 to provide 100 (1× minimum recommended dose), 250 or 2,500 (25×) FTU/kg feed. Enzyme activities were confirmed by analysis; enzyme activities in the 2,500 FTU/kg group were higher than intended, 3,430 and 3,270 FTU/kg feed for gestating and lactation diets, respectively. The gestation diet was offered in mash form and sows were restrictively fed until day 85 of gestation and thereafter ad libitum until farrowing. Then, the lactation diet was offered in mash form and on ad libitum basis. Health status and mortality of sows and piglets were monitored throughout the study. Sow’s weight was measured at the start of the experiment, on day 109 of gestation and at weaning and their feed intake was recorded daily (group fed in the gestation). At farrowing, the number of piglets born and the body weight were recorded. Litter weight was also measured on days 21 and 28 of lactation. On the last day of study (day 28 of lactation), blood samples were obtained from sows for routine blood haematology and biochemistry. An ANOVA was done with the data and group means were compared with Tukey test.

No sows died during the study. Body weight and body weight change of the sows were not different between treatments. Feed intake during lactation was 3.68, 3.74, 4.33 and 4.17 kg/day for control, 100, 250 or 2,500 FTU/kg feed, the values of 250 and 2,500 FTU/kg groups were significantly higher than the control group. On average, litter size on day 1 was 12, litter size at weaning was 10.6, and the litter weight on day 1 was 18.4 kg and at weaning was 77.9 kg. There were no significant differences between treatments in any of the parameters studied including blood parameters (except enzyme activities in the 2,500 FTU/kg group were higher than intended).
3.2.2.6. Conclusions on safety for the target species

The results of the tolerance trials showed that chickens and turkeys for fattening tolerated 400-fold the minimum recommended dose, laying hens 250-fold, weaned piglets 500-fold and sows 25-fold. Therefore, the FEEDAP Panel concludes that the additive is safe at the respective minimum recommended levels, namely chickens for fattening 125 FTU/kg feed, turkeys for fattening 250 FTU/kg feed, laying hens 200 FTU/kg feed and piglets and sows at 100 FTU/kg feed, with a wide margin of safety.

The conclusion reached in chickens and turkeys for fattening can be extended to chickens reared for laying and to turkeys reared for breeding at the corresponding dose and that reached in piglets can be extended to pigs for fattening.

Considering the margin of safety shown in the tolerance trials, the FEEDAP Panel concludes that the additive is safe for minor poultry species and other avian species for fattening or up to the point of lay at the dose of 250 FTU/kg feed, for minor poultry species and other avian species for laying at 200 FTU/kg feed and for other porcine species at 100 FTU/kg feed.

The Panel considers that the different formulations of the additive are equivalent in terms of safety for the target species.

3.2.3. Safety for the consumer

3.2.3.1. Acute oral toxicity study

The applicant provided the results of an acute toxicity study in rats with the liquid concentrate conducted according to OECD TG 423 (201; Acute toxic class method).

3.2.3.2. Genotoxicity and mutagenicity studies

**Bacterial reverse mutation tests**

The liquid and the solid concentrate (spray-dried) were tested for mutagenicity in the bacterial reverse mutation test in Salmonella Typhimurium strains TA 1535, TA 100, TA 1537, TA 98 and in Escherichia coli WP2 uvrA with and without metabolic activation (liver S9 mix from induced rats) according to OECD guideline 471. The top concentrations tested were 5,500 µg/plate for the liquid and 11,000 µg/plate for the solid concentrate. Both the standard plate test and the pre-incubation test were used. No precipitation of the test item was observed. A weak bacteriotoxic effect (slight decrease in the number of his+ revertants) was observed with the solid concentrate in the standard plate test on the tester strain TA 1537 without S9 mix at a concentration of 11,000 µg/plate only. No other sign of bacterial toxicity was reported in any other experimental condition. Both the fermentation products tested negative with all the bacterial strain, with or without metabolic activation, while the positive controls performed as expected.

**In vitro chromosome aberration test**

The liquid concentrate, dissolved in deionised water, was assessed for its potential to induce structural chromosome aberrations in Chinese hamster V79 cells with and without metabolic activation, up to a maximum concentration of 5,000 µg/mL in compliance with OECD guideline 473. Cells were exposed to the test item for 4 h and harvested 14 h later. At least 100 metaphases per culture (200 per experimental point) were evaluated for structural chromosome aberrations. Moderate cytotoxicity was observed only after treatment with 5,000 µg/mL in the presence of S9 mix (maximum reduction of mitotic index: 73.8% of control). Neither precipitation nor relevant in influence on osmolarity or pH value was observed. In the absence of S9 mix, no relevant increase in chromosomal aberrations was observed at the concentrations evaluated. In the presence of S9 mix, significant increases in chromosomal aberrations (excluding gaps) above the solvent control value (2.5%) were reported at

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54 Technical dossier FAD-2014-0044/Section III/Annex III.6 and Supplementary information February 2017/Annex 41.
55 Technical dossier FAD-2014-0044/Section III/Annex III.7 and Supplementary information February 2017/Annex 41.
56 Technical dossier FAD-2014-0044/Section III/Annex III.8 and Supplementary information February 2017/Annex 42.
57 Technical dossier FAD-2014-0044/Section III/Annex III.9 and Supplementary information February 2017/Annex 41.
concentrations of 1,250 and 2,500 µg/mL (5.5% and 8.8%, respectively). Also, in the confirmatory experiment in the presence of S9 mix, the numbers of aberrant cells (excluding gaps) significantly exceeded the control value (3.0%) after treatment with 937.5, 1,875 and 3,750 µg/mL (10.5%, 11.5% and 8.5%, respectively). All these values were clearly above the data of the historical solvent control (0.0–4.0% aberrant cells, excluding gaps). The Panel concluded that the test item is clastogenic in the presence of metabolic activation, in line with the conclusions from the authors of the study.

In vivo micronucleus studies

The liquid concentrate was assessed in NMRI mice (5 animals/group) in an oral (gavage) micronucleus test up to a maximum dose level of 2,000 mg/kg, in compliance with OECD guideline 474.\textsuperscript{58} Samples were prepared 24 and 48 h after administration in the highest dose group of 2,000 mg/kg bw and in the vehicle controls. In the intermediate dose levels and in the positive control groups, the 24-h sacrifice interval was investigated only. Two thousand polychromatic erythrocytes per animal were investigated for micronuclei. No relevant increase in the number of polychromatic erythrocytes containing micronuclei was observed, while the positive control substances led to a significant induction of micronuclei. However, no relevant inhibition of erythropoiesis was reported, therefore the exposure of target cells to the test item was not demonstrated.

Two further in vivo micronucleus assays were submitted in which the test items were administered via intraperitoneal (i.p.) route to NMRI male mice: the liquid concentrate was tested in the first of these studies,\textsuperscript{59} the solid concentrate (spray-dried) in the second one.\textsuperscript{60} Both tests were conducted in compliance with OECD guideline 474. Also, in these two i.p. studies, five male animals per group were treated and samples were prepared 24 and 48 h after administration in the highest dose group and in the vehicle controls, while only the 24-h sacrifice interval was investigated in the intermediate dose levels and in the positive control groups. The maximum administered dose level was 2,000 mg/kg bw in the first i.p. study, while in the second one, on the basis of a preliminary dose finding test, 1,100 mg/kg bw test material (corresponding to 1,000 mg dry matter/kg bw) was administered as top dosage, at which a slight non-significant reduction of erythropoiesis was reported. In both studies, 2,000 polychromatic erythrocytes per animal were investigated for micronuclei. The positive controls performed as expected. No genotoxic effect was reported in any of these two studies. However, considering that the in vitro positive result was reported only after metabolic activation and that the i.p. administration does not warrant that the test item undergoes hepatic metabolism before reaching the target, the two i.p. studies cannot be considered sufficient to rule out the potential in vivo genotoxicity of the fermentation product.

In vivo comet assay

An in vivo comet assay in Wistar Han rat was performed with the liquid concentrate used to formulate the additive. Only male animals were used because a preliminary test revealed no sex-related differences in the response to the test item. Groups of five rats per dose level were treated dosed twice by oral gavage with water or with 250, 500, 1,000 and 2,000 mg liquid concentrate per kg bw for two consecutive days, in compliance with OECD guideline 489.\textsuperscript{61} A positive control group was dosed twice by oral gavage with 200 mg ethyl methane sulfonate (EMS) per kg bw.

No treatment-related clinical signs or mortality were noted. The animals were sacrificed by abdominal aorta bleeding under isoflurane anaesthesia approximately 3–4 h after the second dose of EMS, water or the test item and the liver was isolated. Single cell suspensions from the liver were made followed by slide preparation. The slides were analysed and the tail intensity (%) was assessed. The mean tail intensity in liver cells of male vehicle-treated rats was moderate (1.68%) indicating a low DNA damage level occurring spontaneously or induced by the experimental procedure.

No statistically significant increase in the mean tail intensity (%) was observed in liver cells of male rats treated with the liquid concentrate compared to the vehicle treated animals (the tail intensity in positive EMS control was 92.33%). Histopathological analysis revealed no treatment-related effect in liver.

\textsuperscript{58} Technical dossier FAD-2014-0044/Section III/Annex III.10 and Supplementary information February 2017/Annex 41.

\textsuperscript{59} Technical dossier FAD-2014-0044/Section III/Annex III.11 and Supplementary information February 2017/Annex 41.

\textsuperscript{60} Technical dossier FAD-2014-0044/Section III/Annex III.12 and Supplementary information February 2017/Annex 42.

\textsuperscript{61} Technical dossier/Supplementary information September 2017/Annex 1.
Conclusions on the genotoxicity

The fermentation product showed clastogenic effects in vitro only in the presence of metabolic activation. No genotoxic effect was reported in vivo in three micronucleus studies, one oral and two i.p., although these studies could not be considered conclusive. However, an in vivo comet assay in liver after oral administration demonstrated that there is no concern for in vivo genotoxicity.

3.2.3.3. Subchronic oral toxicity study

The applicant provided a 90-day study in rats conducted on liquid concentrate according to OECD TG 408 (1998). Groups of 10 Crl:WI(Han) rats of each sex received diets containing 0, 1,500, 5,000 or 15,000 mg/kg of phytase concentrate for 13 weeks. Clinical observations were performed before the first treatment and daily thereafter. A full clinical examination was performed once a week. The study was conducted with the full range of observations required by the guideline, including functional and neurobehavioural parameters, body weight, food consumption, ophthalmology, haematology, clinical chemistry and urine analysis. Selected organs were weighed, fixed and preserved at necropsy and examined microscopically for histological changes.

There were no treatment-related effects on clinical observations, body weight, water or feed intake. No treatment-related differences were seen in the results of the functional observational battery, urinalysis or ophthalmoscopic examination.

Some small significant differences were seen in the results of haematological examination (increased red blood cell counts in two highest male groups; reduced relative monocyte counts in intermediate female group; increased large unstained cell counts in high-dose females). These differences were within the range of historic values and are considered incidental and unrelated to treatment. Results of clinical chemistry measurements showed some small significant differences (alanine aminotransferase and aspartate aminotransferase levels reduced in intermediate-dose males; reduced albumin levels in low- and intermediate-dose females; higher urea levels in high-dose males). The differences seen were either unrelated to dose or within the historical control range, thus are considered to be incidental and unrelated to treatment.

Compared to controls, mean brain weight was higher in low-dose animals of both sexes and in intermediate-dose females. Spleen weight was higher than that of controls in low-dose females and relative spleen weight was higher than controls for both sexes. The lack of dose relationship of these differences indicates that they are most likely to be incidental findings unrelated to treatment.

Histological examination showed a higher incidence of renal abnormalities (basophilic tubules) in both sexes of rats from the highest dose group compared with the controls (9/10 and 7/10 compared with 5/10 and 4/10 for males and females, respectively). The differences are not statistically significant and although they may represent normal variation of a common lesion they cannot be entirely dismissed as possibly related to treatment. No such effect was observed at the intermediate dose of 5,000 mg/kg feed.

3.2.3.4. Conclusions on safety for the consumer

The results obtained with the enzyme concentrates in the genotoxicity studies and in the subchronic oral toxicity study do not indicate any reason for concern for consumer safety arising from the use of the product as feed additive. Therefore, the Panel considers that the additive is safe for the consumers of food products derived from animals fed with the additive.

3.2.4. Safety for the user

3.2.4.1. Acute inhalation toxicity study

An acute inhalation toxicity study was performed with solid concentrate (spray-dried) at an analysed concentration of 5.20 mg/L according to OECD TG 403. Five rats of each sex were exposed nose only to the test atmosphere for a period of 4 h and then monitored for 14 days. There was no mortality but clinical observation noted laboured respiration during exposure which persisted to a slight level for up to 4 days after exposure. No abnormalities of clinical condition were noted from 5 days after exposure. Body weight for both sexes decreased during the first few days after exposure but increased for males from day 3 and for females from day 7. No abnormalities were identified at necropsy.

62 Technical dossier FAD-2014-0044/Section III/Annex III.13 and Supplementary information February 2017/Annex 41.
63 Technical dossier FAD-2014-0044/Section III/Annex III.14.
3.2.4.2. Effects on the eyes and skin

A skin irritation test was performed according to OECD Guideline 404 in three New Zealand White rabbits using the solid concentrate (spray-dried). No oedema was observed in any of the animals. Very slight erythema was observed in two animals and well defined erythema was observed in the third. In all cases, the cutaneous reactions were reversible within 72 h after the removal of the applied patch. Therefore, the concentrate is considered to be non-irritant to the skin.

An eye irritation test was performed according to OECD Guideline 405 in three New Zealand White rabbits using the solid concentrate (spray-dried). No reactions were noted in the cornea nor in the iris of the animals. Findings were limited to slight conjunctival redness and discharge, which were in all cases reversible within 24 h. Therefore, the concentrate is considered to be non-irritant to eyes.

Dermal sensitisation was investigated in a local lymph-node assay according to OECD Guideline 442 in female mice and using the solid concentrate (spray-dried) which showed that the product has the potential to be a dermal sensitiser. The applicant sent a paper on the immunoglobulin E (IgE)-mediated allergy to the phytase in subjects exposed to the product, demonstrating that it is highly sensitising.

3.2.4.3. Conclusions on safety for the user

The solid concentrate was not toxic by inhalation, not irritant to skin or eyes but showed a potential to be a dermal sensitiser, which has been demonstrated in exposed workers. No data were submitted for the liquid concentrate. Considering the composition of the final formulations, the Panel concludes that the conclusions reached for the solid concentrate apply to the final formulations of the additive. Owing to the proteinaceous nature of the active substance, the FEEDAP Panel considers that the additive is also to be considered a potential respiratory sensitiser.

3.2.5. Safety for the environment

Neither the production strain nor its recombinant DNA was detected in the final product. The final product does not pose any environmental safety concern associated with the genetic modification. The active substance of the additive is a protein, and as such will be degraded/inactivated during passage through the digestive tract of animals or in the environment. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

3.3. Efficacy

In all the studies presented in this section, the significance level was set at $p < 0.05$.

3.3.1. Efficacy for chickens for fattening

Two short-term trials and five long-term trials were evaluated. Two of these trials were not considered in the assessment. One of the short-term trials was not considered because measurements included only performance of the birds and bone mineralisation. The Panel considers that the bone mineralisation should be coupled with an indication of the digestibility. However, the Panel notes that the study provided supporting evidence on the equivalence of the granular and powder forms regarding the mineralisation of the bones. The other study that was not considered was a long-term trial in which a high mortality was registered. Mortality was 27%, 16%, 12% and 6% for control, 125, 250 FTU/kg feed and positive control, respectively. The mortality was mostly found in the grower and finisher periods and dead birds showed growth retardation and leg problems.

3.3.1.1. Short-term trial

A total of 384 seven-day-old male chickens for fattening (Ross 308) were caged in groups of four birds and allocated to eight dietary treatments (representing 12 replicates per treatment). A basal diet based on maize and soybean meal (total phosphorus content of 0.39%; calcium content of

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64 Technical dossier FAD-2014-0044/Section III/Annex III.15.
65 Technical dossier FAD-2014-0044/Section III/Annex III.16.
66 Technical dossier FAD-2014-0044/Section III/Annex III.17.
67 Technical dossier FAD-2014-0044/Section III/Annex III.18.
68 Technical dossier FAD-2015-0040/Annex IV.23.
69 Technical dossier/Supplementary information February 2017/Annex 13.
70 Technical dossier FAD-2014-0044/Section IV/Annex IV.13 and Supplementary information February 2017.
0.48%) was either not supplemented (control) or supplemented with Natuphos® E 10000 L to provide 125, 250, 375, 500, 625 and 750 FTU/kg feed. A positive control was also considered (total phosphorus content of 0.74%; calcium content of 0.90%). Feed was offered *ad libitum* in pelleted form from day 7 to day 21 and presented titanium dioxide as an external marker. Enzyme activities were confirmed by analysis. Excreta samples were collected during three consecutive days starting on day 18, pooled per cage and analysed to study phosphorus retention. On day 21, the birds were killed and samples of the ileal digesta were collected and pooled per cage and analysed to study phosphorus utilisation. The tibia from one of the sacrificed chickens per cage was collected and mineralisation of the bone was studied. An ANOVA was performed with the data obtained and group means were compared with the Duncan test. The results are presented in Table 1. Birds fed the phytase showed a significantly higher ash and phosphorus content in tibia bone from the dose of 250 FTU/kg feed. However, there were no effects of the phytase on the ileal digestibility of phosphorus and significant increases in phosphorus retention were found only at the dose of 750 FTU/kg feed.

### Table 1: Effect of Natuphos® E on apparent ileal phosphorus digestibility, phosphorus retention and bone mineralisation in chickens for fattening

| Group FTU/kg feed | Phosphorus (%) | Tibia content(%)<sup>1</sup> |
|-------------------|----------------|-----------------------------|
|                   | Ileal digestibility | Retention | Ash | Phosphorus |
| Control           | 63.6            | 81.2<sup>bc</sup> | 38.2<sup>e</sup> | 5.7<sup>d</sup> |
| 125               | 63.5            | 80.4<sup>c</sup> | 40.8<sup>d</sup> | 6.1<sup>cd</sup> |
| 250               | 67.4            | 82.4<sup>bc</sup> | 41.4<sup>cd</sup> | 6.4<sup>bc</sup> |
| 375               | 68.5            | 81.6<sup>bc</sup> | 41.1<sup>cd</sup> | 6.2<sup>c</sup> |
| 500               | 64.1            | 81.3<sup>bc</sup> | 42.9<sup>bc</sup> | 6.7<sup>bc</sup> |
| 625               | 66.1            | 86.4<sup>ab</sup> | 42.9<sup>bc</sup> | 6.7<sup>ab</sup> |
| 750               | 70.2            | 88.0<sup>a</sup> | 44.5<sup>b</sup> | 6.9<sup>a</sup> |
| Positive control  | 70.6            | 57.7<sup>d</sup> | 47.8<sup>a</sup> | 7.0<sup>a</sup> |

<sup>1</sup> Reported as % in dry bone.

<sup>a,b,c,d,e</sup>: Values within one column with different superscripts are significantly different (p < 0.05).

#### 3.3.1.2. Long-term trials

The first long-term trial is the tolerance trial presented in Section 3.2.2.1. The performance of the birds was measured along with measurements on the phosphorus utilisation and tibia mineralisation. For the measurement on the utilisation of phosphorus on day 43 of life five birds per pen were selected randomly and killed, samples of the ileal and cloacal contents were collected. Samples collected from birds of the same pen were pooled and analysed. On the same day, the left and right tibia bones from three birds per pen were collected to study the bone mineralisation and breaking strength.

In the second trial, a total of 1,920 one-day-old male chickens (Ross 308) were distributed to pens in groups of 24 birds and allocated to eight groups (representing 10 replicates per treatment). Two basal diets, starter and grower, based on maize and soybean meal (total phosphorus content 0.47/0.44%; calcium content 0.59/0.51%) were either not supplemented (control) or supplemented with Natuphos® E 5000 to provide 125, 250, 375, 500, 750 or 1,000 FTU/kg feed. Enzyme activities were confirmed by analysis. A positive control (total phosphorus content 0.71% and 0.63%; calcium 0.92% and 0.75%) was also considered. Diets were offered *ad libitum* in the form of coarse meal for 38 days. Health status of the birds was monitored throughout the study. Birds were weighed and feed intake was measured. Feed to gain ratio was calculated. On the third and fifth week under study, one bird per pen was caged individually to do a balance study, which consisted of four days of adaptation to the diet with the external marker and five days of excreta collection. At the end of the balance, the birds were killed and tibia bones were collected to study the mineralisation and bone strength.

The third and the fourth long-term trials shared the same study design; one was done with males and the other one with females. In each trial, a total of 960 one-day-old chickens (Ross 308) were distributed to pens in groups of 20 birds and allocated to three dietary treatments (representing 16
replicates per treatment). Three basal diets, starter, grower and finisher, based on maize and soybean meal (total phosphorus content 0.62% to 0.44%; calcium content 0.87% to 0.61%) were either not supplemented (control) or supplemented with Natuphos® E 5000 L to provide 125 FTU/kg feed (confirmed by analysis). A positive control (total phosphorus content 0.66% to 0.56%; calcium 0.92% to 0.78%) was also considered. Diets were offered ad libitum in pelleted form for 35 days. Health status of the birds was monitored throughout the study. Birds were weighed and feed intake registered. Feed to gain ratio was calculated.

The data from each of the three studies was analysed with an ANOVA and the group means were compared with Tukey test. The results on the performance and mortality of the birds are presented in Table 2, the results on the phosphorus availability and tibia mineralisation are shown in Table 3.

Final body weight was higher in the chickens for fattening receiving the phytase compared to the control diet in three trials at the dose of 50,000 FTU/kg feed in trial 1, from 250 FTU/kg in trial 2 and at 125 FTU/kg feed in trials 3 and 4. The Panel notes that trials 3 and 4 were conducted in the same trial site, same dates and using the same diets, the only difference was that trial 3 reports the data for males and trial 4 reports data for females. Therefore, the Panel cannot consider trials 3 and 4 as independent trials and will be considered as a single study.

The results on the phosphorus utilisation (Table 3) from the long-term trials showed improvements on the retention of phosphorus at 50,000 FTU/kg feed in trial 1 and at 125 in trial 2 (grower phase); however, in the latter, the effect on the retention was not found in dosages of 375 FTU/kg or above. In trial 2, there were significant increases on the ileal digestibility and bone mineralisation from the dose of 250 FTU/kg feed.

In summary, performance of the birds was higher compared to the control in three trials, one at the dose of 50,000 FTU/kg, one at 250 FTU/kg and another one at 125 FTU/kg feed, in two of these trials improvements on the phosphorus utilisation were found at 50,000 and at 250 FTU/kg feed. In a further short-term trial, phosphorus retention was increased at 750 FTU/kg feed. Based on the results

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Table 2: Effect of Natuphos® E on the performance and mortality in chickens for fattening

| Trial | Group FTU/kg feed | Daily feed intake (g) | Final body weight (g) | Feed to gain ratio | Mortality/ culling (%) |
|-------|-------------------|-----------------------|-----------------------|--------------------|-----------------------|
| 1\(^1\) | Control 124\(^c\) | 3,303\(^a\) | 1.59 | 5.0/1.1 |
|       | 125 127\(^bc\) | 3,404\(^ab\) | 1.59 | 3.9/0.4 |
|       | 250 128\(^ab\) | 3,429\(^ab\) | 1.59 | 2.9/0.7 |
|       | 500 127\(^bc\) | 3,422\(^ab\) | 1.59 | 1.8/0.0 |
|       | 50,000 130\(^ab\) | 3,490\(^a\) | 1.58 | 1.8/0.4 |
|       | Positive control 132\(^a\) | 3,502\(^a\) | 1.61 | 1.4/0.7 |
|       | 125 74\(^d\) | 2,018\(^c\) | 1.48 | 4.6/2.5 |
| 2\(^2\) | Control 77\(^cd\) | 2,148\(^bc\) | 1.47 | 2.1/0.4 |
|       | 250 82\(^bc\) | 2,317\(^ab\) | 1.44 | 4.2/0.4 |
|       | 375 82\(^bc\) | 2,259\(^ab\) | 1.48 | 4.6/3.5 |
|       | 500 81\(^bc\) | 2,263\(^ab\) | 1.47 | 4.2/0.8 |
|       | 750 84\(^ab\) | 2,358\(^a\) | 1.44 | 2.1/0.1 |
|       | 1,000 88\(^a\) | 2,355\(^a\) | 1.49 | 2.9/0.4 |
|       | Positive control 88\(^a\) | 2,413\(^a\) | 1.48 | 2.5/1.3 |
| 3\(^3\) | Control 103\(^a\) | 2,245\(^c\) | 1.63\(^a\) | 7.8 |
|       | 125 106\(^a\) | 2,337\(^ab\) | 1.62\(^a\) | 9.1 |
|       | Positive control 107\(^a\) | 2,410\(^a\) | 1.59\(^b\) | 5.6 |
| 4\(^4\) | Control 94\(^b\) | 2,058\(^b\) | 1.64 | 2.8 |
|       | 125 97\(^a\) | 2,153\(^a\) | 1.62 | 4.7 |
|       | Positive control 98\(^a\) | 2,181\(^a\) | 1.61 | 2.0 |

1: In trials 3 and 4, values include mortality and culling.
2: Technical dossier/Supplementary information February 2017/Annex 36.
3: Technical dossier/Supplementary information February 2017/Annex 37.
4: Values within one trial and within one column with different superscripts are significantly different (p < 0.05).

The results on the phosphorus utilisation (Table 3) from the long-term trials showed improvements on the retention of phosphorus at 50,000 FTU/kg feed in trial 1 and at 125 in trial 2 (grower phase); however, in the latter, the effect on the retention was not found in dosages of 375 FTU/kg or above. In trial 2, there were significant increases on the ileal digestibility and bone mineralisation from the dose of 250 FTU/kg feed.

In summary, performance of the birds was higher compared to the control in three trials, one at the dose of 50,000 FTU/kg, one at 250 FTU/kg and another one at 125 FTU/kg feed, in two of these trials improvements on the phosphorus utilisation were found at 50,000 and at 250 FTU/kg feed. In a further short-term trial, phosphorus retention was increased at 750 FTU/kg feed. Based on the results

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73 Technical dossier/Supplementary information February 2017/Annexes 36 and 37.
obtained in two performance trials and in the short-term trial, the Panel concludes that the additive has a potential to be efficacious to improve the performance and/or the phosphorus utilisation at 750 FTU/kg feed.

The efficacious dose is higher than the one recommended by the applicant. The conclusions on the tolerance were drawn based on the recommended dosage; however, the dosages tested, parameters measured and the results obtained in the tolerance trial allow the Panel to consider that the dose of 750 FTU/kg feed is safe for chickens for fattening.

### 3.3.2. Efficacy for turkeys for fattening

Four short-term trials and two long-term trials were evaluated.

#### 3.3.2.1. Short-term trials

In the first short-term trial, a total of 1,520 one-day-old male turkeys (converter hybrid) were distributed in pens in groups of 20 birds and allocated to six dietary treatments (eight pens for control and six for the other treatments). Two basal diets, starter and grower, based on maize and soybean meal (total phosphorus content 0.70% and 0.60%; calcium content 0.95% and 0.90%) were either not supplemented (negative control) or supplemented with Natuphos® E 5000 to provide 125, 250, 500, 1,000 FTU/kg feed. Enzyme activity was confirmed by analysis. A positive control (total phosphorus content 0.95% and 0.90%; calcium content 1.0% and 0.90%) was also included. Diets were offered ad libitum in pelleted form for 42 days; diets presented titanium dioxide as an external marker. Health status of the birds was monitored throughout the study. A balance study was done on day 38 by individually caging 16 birds per treatment. After 4 days of adaptation to the diet/cage, a 5-day collection followed. On day 42, 32 birds per group were killed in order to collect ileal digesta samples and tibia bones were collected from eight birds.

In the second short-term trial, a total of 216 seven-day-old female turkeys (BUT 9) were caged in groups of three and allocated to six dietary treatments, representing 12 replicates per group. Two basal diets, starter and grower, based on maize and soybean meal (total phosphorus content 0.86% and 0.50%; calcium content 1.20% and 0.70%) were either not supplemented (negative control) or supplemented with Natuphos® E 5000 to provide 125, 250, 500, 750 FTU/kg feed. Enzyme activity was confirmed by analysis. A positive control (total phosphorus content 0.95% and 0.90%)

| Trial | Group FTU/kg feed | Phosphorus (%) | Tibia mineralisation (%)<sup>2</sup> |
|-------|-------------------|----------------|-------------------------------------|
|       |                   | Ileal digestibility | Retention | Ash | Phosphorus | Ash | Phosphorus |
| 1<sup>71</sup> | Control | 51.7<sup>a</sup> | 49.2<sup>b</sup> | 41.6<sup>ab</sup> | 7.0<sup>ab</sup> |
|       | 125 | 64.9<sup>d</sup> | 51.5<sup>ab</sup> | 40.0<sup>b</sup> | 6.7<sup>b</sup> |
|       | 250 | 72.0<sup>c</sup> | 57.4<sup>ab</sup> | 41.5<sup>ab</sup> | 7.0<sup>ab</sup> |
|       | 500 | 77.1<sup>b</sup> | 65.0<sup>ab</sup> | 42.3<sup>ab</sup> | 7.0<sup>ab</sup> |
|       | 50,000 | 93.7<sup>a</sup> | 67.6<sup>a</sup> | 42.5<sup>ab</sup> | 7.2<sup>a</sup> |
|       | Positive control | 70.3<sup>c</sup> | 56.1<sup>ab</sup> | 43.3<sup>a</sup> | 7.3<sup>a</sup> |
| 2<sup>72</sup> | Control | 61.2<sup>cd</sup>/56.1<sup>e</sup> | 62.6<sup>d</sup>/54.1<sup>b</sup> | 48.1<sup>c</sup>/51.1<sup>c</sup> | 7.9<sup>e</sup>/8.4<sup>e</sup> |
|       | 125 | 62.7<sup>d</sup>/62.5<sup>de</sup> | 63.0<sup>e</sup>/61.2<sup>c</sup> | 50.1<sup>de</sup>/52.2<sup>b</sup> | 8.3<sup>de</sup>/8.5<sup>bc</sup> |
|       | 250 | 62.1<sup>cd</sup>/66.4<sup>cd</sup> | 62.1<sup>d</sup>/60.4<sup>a</sup> | 52.0<sup>d</sup>/50.4<sup>c</sup> | 8.6<sup>cd</sup>/8.4<sup>c</sup> |
|       | 375 | 64.1<sup>c</sup>/69.1<sup>bcd</sup> | 63.7<sup>c</sup>/60.1<sup>ab</sup> | 53.6<sup>b</sup>/54.0<sup>abc</sup> | 9.0<sup>abc</sup>/9.0<sup>abc</sup> |
|       | 500 | 65.0<sup>d</sup>/70.6<sup>bc</sup> | 63.9<sup>c</sup>/58.5<sup>ab</sup> | 53.3<sup>c</sup>/55.6<sup>ab</sup> | 8.9<sup>de</sup>/9.2<sup>bc</sup> |
|       | 750 | 70.3<sup>d</sup>/74.8<sup>ab</sup> | 61.5<sup>d</sup>/58.6<sup>ab</sup> | 55.0<sup>d</sup>/55.7<sup>ab</sup> | 9.3<sup>de</sup>/9.3<sup>ab</sup> |
|       | 1,000 | 71.9<sup>c</sup>/78.2<sup>a</sup> | 62.5<sup>c</sup>/59.8<sup>ab</sup> | 55.5<sup>c</sup>/55.8<sup>ab</sup> | 9.4<sup>c</sup>/9.2<sup>ab</sup> |
|       | Positive control | 56.0<sup>c</sup>/55.4<sup>e</sup> | 44.9<sup>d</sup>/41.4<sup>c</sup> | 56.7<sup>a</sup>/56.6<sup>a</sup> | 9.5<sup>a</sup>/9.6<sup>a</sup> |

1: Values in trial 2 are for starter/grower period, respectively.
2: In trial 1, values reported as per cent in dry bone, and in trial 2, values reported as per cent in fat free dry matter.

<sup>a,b,c,d,e</sup>: Values within one trial and within one column with different superscripts are significantly different (p < 0.05).
calcium content 1.26% and 1.05%) was also included. Diets were offered *ad libitum* in mash/pelleted form for 49 days; diets presented titanium dioxide as an external marker. Health status of the birds was monitored throughout the study. Balance studies were performed on day 26 and 47 of age; samples of excreta were collected for 3 days. At the end of the study, all animals were killed in order to collect ileal digesta samples. Tibia bones from 12 birds were also collected.

The third and fourth short-term trials were replicate studies which shared a common design and feed. In each of them, a total of 504 one-day-old male turkeys (converter hybrid) were distributed to pens in groups of 18 birds and allocated to four dietary treatments, representing seven replicates per treatment.76 Three basal diets, starter, grower and finisher based on maize and soybean meal (total phosphorus content from 0.70% to 0.43%; calcium content 1.0% to 0.95%) were either not supplemented (negative control) or supplemented with Natuphos® E 5000 to provide 125 FTU/kg feed (confirmed by analysis). A positive control (total phosphorus content from 1.0% to 0.77%; calcium content 1.3% to 1.0%) was also included. Diets were offered *ad libitum* in pelleted form for 63 days; diets presented titanium dioxide as an external marker. Health status of the birds was monitored throughout the study. Balance studies were performed on days 38-44 and days 57-63 of age by individually caging 12 birds per treatment. After 4 days of adaptation, a 7-day collection followed. On day 42 of life, eight birds per treatment were killed and tibia bones were collected to study bone mineralisation.

In the four trials, an ANOVA was done with the data and group means were compared with Tukey or Dunnett test. The results of the short-term trials are presented in Table 4. Results showed increases in the phosphorus retention from 125 FTU/kg feed in all trials.

**Table 4:** Effect of Natuphos® E on apparent ileal phosphorus digestibility, phosphorus retention and bone mineralisation in turkeys for fattening

| Trial | Group FTU/kg feed | Phosphorus (%) | Tibia content (%)<sup>2</sup> |
|-------|-----------------|----------------|---------------------------|
|       |                 | Ileal digestibility | Retention<sup>1</sup> | Ash | Phosphorus |
| 38 days |                   |                 |                         |     |           |
| 1<sup>74</sup> | Control | 51.3<sup>c</sup> | 55.0<sup>c</sup> | – | 38.2<sup>d</sup> | 6.3<sup>d</sup> |
|       | 125       | 53.6<sup>bc</sup> | 63.3<sup>ab</sup> | – | 39.1<sup>cd</sup> | 6.5<sup>cd</sup> |
|       | 250       | 61.3<sup>a</sup>  | 65.5<sup>a</sup>  | – | 39.9<sup>bc</sup> | 6.6<sup>bcd</sup> |
|       | 500       | 60.5<sup>a</sup>  | 63.2<sup>ab</sup> | – | 40.6<sup>bc</sup> | 6.8<sup>bc</sup> |
|       | 1,000     | 61.7<sup>a</sup>  | 62.1<sup>b</sup>  | – | 41.1<sup>ab</sup> | 6.8<sup>b</sup> |
|       | Positive control | 57.3<sup>ab</sup> | 41.0<sup>d</sup> | – | 42.7<sup>a</sup> | 7.4<sup>a</sup> |
|       | 26-28 days |                     |                         |     |           |
|       | 47-49 days |                     |                         |     |           |
| 2<sup>75</sup> | Control | 50.6 | 37.8 | 49.0 | 48.3 | – |
|       | 125       | 53.7 | 44.2* | 51.2 | 49.8 | – |
|       | 250       | 56.6* | 39.8 | 52.3 | 49.3 | – |
|       | 500       | 59.8* | 42.2 | 54.2 | 50.9* | – |
|       | 750       | 69.4* | 44.6* | 59.0* | 50.4* | – |
|       | Positive control | 51.1 | 34.8 | 38.5 | 51.6* | – |
|       | 38 days |                     |                         |     |           |
|       | 57 days |                     |                         |     |           |
| 3<sup>3</sup> | Control | 49.5 | 59.6 | 52.4 | – |
|       | 125       | 55.0* | 65.9* | 56.5 | – |
|       | 250       | 57.5* | 67.7* | 57.7 | – |
|       | Positive control | 48.5 | 41.0* | 72.4* | – |

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<sup>76</sup> Technical dossier FAD-2014-0044/Supplementary information February 2017/Annexes 58 and 59.
3.3.2.2 Long-term trials

The first long-term trial is the tolerance trial presented in Section 3.2.2.2.77 In that study, performance of the birds was studied together with measurement on the phosphorus utilisation. Ileal digestibility of phosphorus and retention was studied in two periods. To study the ileal digestibility of phosphorus, 188 birds were selected from the pens (four birds per pen) and were killed to collect ileal samples. The balance studies were done from day 17 to day 24 and from 39 to 46: one bird per pen was selected and kept in a cage for 8 days, after a 4-day adaptation period, a 4-day collection period followed.

The second long-term trial was done with total of 1,200 one-day-old female turkeys (BUT 10) were distributed to pens in groups of 25 birds and allocated to six dietary treatments (representing eight replicates per treatment).78 Four basal diets, starter and growers, based on maize and soybean meal (total phosphorus content from 0.73% to 0.40%; calcium content 1.02% to 0.56%) were either not supplemented (control) or supplemented with Natuphos® E 5000 L to provide 125, 250, 500 or 1,000 FTU/kg feed. Enzyme activity was confirmed by analysis. A positive control (total phosphorus content 0.92% to 0.62%; calcium content 1.29% to 0.87%) was also included. Diets were offered ad libitum in mash or pelleted form for 96 days. Health and mortality were monitored. Feed intake and body weight were measured throughout the study. Feed to gain ratio was calculated. At the end of the study, two birds per pen were killed and tibia bone was collected to study the bone mineralisation. An ANOVA was done with the data and mean groups were compared using the Duncan test.

The results on the performance of the turkeys are presented in Table 5 and the effects on the utilisation of phosphorus from the first long-term trial are presented in Table 6.

### Table 5: Effect of Natuphos® E on the performance of turkeys for fattening

| Trial | Group FTU/kg feed | Daily feed intake (g) | Final body weight (kg) | Feed to gain | Mortality/culling |
|-------|-------------------|-----------------------|------------------------|--------------|------------------|
| 1     | Control           | 212                   | 10.0<sup>b</sup>c      | 2.28         | 1.9/0.4          |
|       | 250               | 209                   | 9.95<sup>c</sup>       | 2.28         | 3.2/0.9          |
|       | 500               | 212                   | 10.1<sup>abc</sup>     | 2.31         | 5.6/0.9          |
|       | 1,000             | 210                   | 10.3<sup>ab</sup>      | 2.24         | 3.7/3.2          |
|       | Positive control  | 219                   | 10.4<sup>a</sup>       | 2.32         | 2.8/3.2          |
| 2     | Control           | 211<sup>b</sup>       | 8.6<sup>c</sup>        | 2.38<sup>a</sup> | 1.1/0.6        |
|       | 125               | 219<sup>ab</sup>      | 8.9<sup>b</sup>        | 2.38<sup>a</sup> | 3.1/1.5        |
|       | 250               | 217<sup>ab</sup>      | 9.0<sup>ab</sup>       | 2.32<sup>ab</sup> | 3.4/2.5        |
|       | 500               | 221<sup>a</sup>       | 9.1<sup>ab</sup>       | 2.34<sup>ab</sup> | 1.5/2.0        |
|       | 1,000             | 224<sup>a</sup>       | 9.0<sup>ab</sup>       | 2.39<sup>a</sup> | 0.5/2.5        |
|       | Positive control  | 218<sup>ab</sup>      | 9.2<sup>a</sup>        | 2.28<sup>a</sup> | 0.5/0.5        |

a,b,c: Values within one trial and within one column with different superscripts are significantly different (p < 0.05).

77 Technical dossier FAD-2014-0044/Section IV/Annex 14 and Supplementary information February 2017/Annex 32.
78 Technical dossier FAD-2014-0044/Section IV/Annex IV.16 and Supplementary information February 2017/Annex 39.
In trial 2, the birds receiving the phytase from 250 FTU/kg feed had a significantly higher final body weight compared to the control. In trial 1, no modifications on the performance of the birds were found. However, birds fed 250 FTU/kg feed showed a higher apparent ileal digestibility of phosphorus (with no data on the bone) compared to control and an increase in the retention of phosphorus was found in birds fed 500 FTU/kg feed in the starter phase compared to control. In the second study, tibia ash content was 42.9%, 46.7%, 44.9%, 45.4%, 43.6% and 44.0% for control, 125, 250, 500, 1,000 FTU/kg and positive control, respectively, and P content was 6.7%, 7.7%, 7.3%, 7.3%, 7.1% and 7.2%. Significant increases on phosphorus content in bone were found from 125 FTU/kg compared to control. However, the data on the bone mineralisation was not accompanied with data on the digestibility.

Table 6: Effect of Natuphos® E apparent ileal digestibility of phosphorus and retention in turkeys for fattening in the first long-term trial

| Group FTU/kg feed | Ileal digestibility of phosphorus | Retention of phosphorus |
|-------------------|----------------------------------|------------------------|
|                   | 24 days  | 42 days  | 17–24 days | 39–46 days |
| Control           | Control  | Control  | Control    | Control    |           |
|                   | 54.6b    | 56.4c    | 52.5b      | 47.5a      |           |
| 250               | 58.4a    | 60.8bc   | 54.2ab     | 51.6a      |           |
| 500               | 58.8a    | 66.6abc  | 57.4a      | 51.8a      |           |
| 1,000             | 61.0a    | 68.2a    | 56.9a      | 50.3a      |           |
| Positive control  | 53.8b    | 57.0c    | 45.6c      | 31.8c      |           |

a,b,c: Values within one column with different superscripts are significantly different (p ≤ 0.05).

In summary, the results of the four short-term trials and one long-term trial showed that the birds receiving the phytase at 125 FTU/kg feed had a higher phosphorus retention compared to control. Therefore, the Panel concludes that the additive has the potential to be efficacious for turkeys for fattening at a minimum dose of 125 FTU/kg feed.

3.3.3. Efficacy for laying hens

Three short-term trials and three long-term trials were evaluated.

3.3.3.1. Short-term trials

In the first short-term trial, a total of 180 Hy-Line-Brown hens 19-weeks-old were caged in groups of three hens and allocated to five dietary treatments (representing 12 replicates per treatment). A basal diet based on maize and soybean meal (total phosphorus content: 0.31%; calcium content 3.4%) was either not supplemented (control) or supplemented with Natuphos® E 5000 in order to provide 0, 100, 200, 300 FTU/kg feed. Enzyme activities were confirmed by analysis. A positive control diet was also considered (total phosphorus content 0.49%; calcium content 3.4%). Feed was offered ad libitum in coarse meal form for 91 days. Health status was monitored throughout the study period. Laying performance (laid eggs and weight) and feed intake were monitored throughout the study and feed to egg mass ratio was calculated. Every 4 weeks, egg-shell thickness and strength were measured on 30 eggs per treatment and the Haugh units every 2 weeks on 10 eggs per treatment. On days 84–91, a balance trial was performed with 24 hen per treatment (8 cages per treatment). Hens were selected and fed the same diet with the difference that the external marker titanium dioxide was present. After 4 days being fed with the diets containing the marker, a 4-day collection period took place. During the balance period, egg mass produced was registered. On day 91, one hen per cage of those involved in the retention study was killed and the ileal contents and tibia bone were collected. Ileal digesta and excreta were analysed to study the utilisation of phosphorus. Tibia bones were analysed for breaking power and mineralisation. An ANOVA was done with the data and group means were compared with the least significant difference test.

Two more reports were submitted which presented data of the same trial. The information and data were presented in separate reports but as indicated in the reports the trial consisted of two balance periods (separated by 1 week) which were presented separately. The Panel notes that the studies are not independent and will be considered as one study (second short-term trial).

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79 Technical dossier FAD-2014-2044/Section IV/Annex IV.19.
80 Technical dossier FAD-2014-0044/Supplementary information February 2017/Annex 62 and 63.
balance period, a total of 152 Tetra-SL hens (21- or 26-week-old) were caged in groups of 2 and allocated to one of the four dietary treatments (representing 19 replicates per treatment). A basal diet based on maize and soybean meal (total phosphorus content 0.34%; calcium content 3.8%) was either not supplemented (negative control) or supplemented with Natuphos® E 5000 G to provide 100 or 200 FTU/kg feed. Enzyme activities were confirmed by analysis. A positive control diet was also considered (total phosphorus content 0.52%; calcium content 3.8%). Diets were offered as mash on ad libitum basis and contained titanium dioxide. After a period of adaptation of 28 days in which body weight, feed intake and laying performance of the hens were measured, excreta was collected for 7 days. At the end of the second balance, one hen per cage was killed and the left tibia was collected for ash, calcium and phosphorus determinations. Eggs laid were weighed and analysed for phosphorus content. An ANOVA was performed with the data and group means were compared with Tukey test.

The results on the apparent ileal phosphorus digestibility, retention, phosphorus content in eggs and tibia are presented in Table 7. The data on the phosphorus utilisation indicated improvements on the retention of phosphorus in all cases from the dose of 100 FTU/kg feed.

In the trials, the data on the feed intake, eggs produced and egg weight indicated no significant differences between the hens fed the phytase and the control group. However, in trial 1, the feed to egg mass ratio showed an increase in hens fed the phytase (2.16, 2.29, 2.23, 2.33 and 2.30 for control, 100, 200, 300 FTU/kg feed and the positive control, respectively). Therefore, in trial 1, the improvement on the phosphorus retention was found concurrently with a negative effect on the feed to egg mass ratio, and consequently, the result on the phosphorus retention cannot support the efficacy of the product. The Panel also notes that in the second short-term trial the egg production during the balance period was probably not studied and this fact would represent a limitation on the data on the phosphorus retention should differences in the production had happened. However, the data in the 4-week period prior to the balance indicated no differences in the eggs laid in the different groups.

### Table 7: Effect of Natuphos® E on phosphorus utilisation in laying hens

| Trial | Group | FTU/kg feed | Phosphorus (%) | Ileal digestibility | Retention | Eggs | Tibia¹ |
|-------|-------|-------------|----------------|---------------------|-----------|------|--------|
| 1     | Control |             |                | 34.4<sup>c</sup>  | 36.7<sup>bc</sup> | –    | 8.6<sup>b</sup> |
|       | 100     |             |                | 37.5<sup>bc</sup> | 41.6<sup>a</sup> | –    | 9.1<sup>b</sup> |
|       | 200     |             |                | 39.4<sup>b</sup>  | 38.6<sup>ab</sup> | –    | 9.1<sup>b</sup> |
|       | 300     |             |                | 39.0<sup>b</sup>  | 40.0<sup>a</sup> | –    | 8.9<sup>b</sup> |
|       | Positive control |             |                | 54.9<sup>a</sup>  | 34.3<sup>c</sup> | –    | 9.8<sup>a</sup> |
| 2a    | Control |             |                | –                  | 19.8<sup>a</sup> | 0.79 | –      |
|       | 100     |             |                | –                  | 27.2<sup>a</sup> | 0.79 | –      |
|       | 200     |             |                | –                  | 27.1<sup>a</sup> | 0.79 | –      |
|       | Positive control |             |                | –                  | 19.8<sup>b</sup> | 0.77 | –      |
| 2b    | Control |             |                | –                  | 21.6<sup>b</sup> | 0.79 | 7.7<sup>b</sup> |
|       | 100     |             |                | –                  | 27.7<sup>a</sup> | 0.79 | 7.7<sup>b</sup> |
|       | 200     |             |                | –                  | 27.2<sup>a</sup> | 0.78 | 8.3<sup>ab</sup> |
|       | Positive control |             |                | –                  | 19.6<sup>b</sup> | 0.79 | 8.7<sup>a</sup> |

1: Values reported as percent of fat free dry matter.

a,b,c: Values within one column with different superscripts are significantly different (p < 0.05).

### 3.3.3.2. Long-term trials

The first long-term trial was the tolerance study presented in Section 3.2.2.3.<sup>81</sup> All groups, except the tolerance group continued the study until completing a 24-week study.

In the second long-term trial, a total of 768 20-week-old Hy-Line Brown hens were caged in groups of eight and allocated to four dietary treatments (representing 24 replicates per treatment).<sup>82</sup> A basal diet based on maize and soybean meal (total phosphorus content 0.35%; calcium content 3.70%) was either not supplemented (control) or supplemented with Natuphos® E 5000 to provide 100 or

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<sup>81</sup> Technical dossier FAD-2014-0044/Section IV/Annex IV.18 and Supplementary information February 2017/Annex 19 and Annex 34.

<sup>82</sup> Technical dossier FAD-2014-0044/Supplementary information February 2017/Annex 30.
200 FTU/kg. Enzyme activities were confirmed by analysis. A positive control diet was also considered (total phosphorus content 0.59%; calcium content 3.70%). Feed was offered ad libitum in mash form from week 20 to week 55 of life.

In the third, a total of 7,920 21-week-old Dekalb White hens were penned in groups of 330 hens and distributed to four dietary treatments (representing six replicates per treatment). A basal diet based on maize and soybean meal (total phosphorus content 0.43%; calcium content 4.0%) was either not supplemented (control) or supplemented with Natuphos® E 5000 to provide 150 or 200 FTU/kg feed. The enzyme activities were confirmed by analysis. A positive control diet was also considered (total phosphorus content 0.60%; calcium content 3.6%). Feed was offered in mash form ad libitum for 24 weeks.

In the three long-term trials feed intake, body weight of the hens and laying performance were monitored throughout the study. Eggs were weighed and feed to egg mass ratio was calculated. In trial 1, phosphorus utilisation was also studied (bone mineralisation and retention). In each trial, an ANOVA was performed with the data and group means were compared with Tukey test. The results on the feed intake, body weight and laying performance of the hens are presented in Table 8 and the results on the phosphorus utilisation in trial 1 are presented in Table 9.

### Table 8: Effect of Natuphos® E on the feed intake, body weight and laying performance of hens

| Trial | Group FTU/kg feed | Daily feed intake (g) | Body weight1 (g) | Egg production2 | Egg weight (g) | Feed to egg mass |
|-------|-------------------|-----------------------|------------------|----------------|----------------|-----------------|
| 1     | Control           | 114                   | 2,006b           | 155            | 62.6           | 1.99            |
|       | 100               | 117                   | 2,082ab          | 157            | 62.0           | 2.01            |
|       | 200               | 116                   | 2,080ab          | 156            | 61.0           | 2.05            |
|       | 300               | 116                   | 2,133a           | 156            | 62.0           | 2.02            |
|       | 500               | 116                   | 2,106ab          | 159            | 62.1           | 2.00            |
|       | Positive control  | 121                   | 2,146a           | 159            | 62.8           | 2.06            |
| 2     | Control           | 111b                  | 371b             | 88.7b          | 62.7b          | 1.99            |
|       | 100               | 112ab                 | 467a             | 90.3ab         | 62.7b          | 1.98            |
|       | 200               | 112ab                 | 461a             | 90.9a          | 62.8ab         | 1.96            |
|       | Positive control  | 114a                  | 501a             | 91.3a          | 63.6a          | 1.96            |
| 3     | Control           | 122                   | 1,639b           | 97.3           | 60.1b          | 2.07            |
|       | 150               | 119                   | 1,731a           | 97.4           | 60.1b          | 2.03            |
|       | 200               | 119                   | 1,712a           | 97.6           | 60.2b          | 2.03            |
|       | Positive control  | 120                   | 1,714a           | 96.9           | 60.5a          | 2.05            |

1: In trials 1 and 3, values are body weight at the end of the study; in trial 2, values are the increase in body weight of the hens during the study.
2: In trial 1, values are the total of eggs laid during the study; in trials 2 and 3, egg production is expressed in percentage.

In all trials, mortality and culling was low (< 2%) with the exception of the control diet in the second long-term trial which reached 6.8%. Mortality was mostly found by the end of the study probably due to the low phosphorus content in the diet. In trial 2, hens fed 200 FTU/kg feed showed a significantly higher egg production compared to the control. No significant effects on the laying performance of the hens were found in trials 1 and 3. In the third study, the data was statistically analysed by comparing the control against the two groups presenting the phytase, the analysis showed a better feed to egg mass ratio in hens receiving the phytase compared to the control; however, the Panel does not consider this as a valid approach.

Phosphorus utilisation in trial 1 showed a significantly higher phosphorus retention in hens fed 500 FTU/kg feed compared to control; however, data on phosphorus content in the bone showed a negative effect (numerical and/or significant) in hens fed the phytase compared to the control.

In summary, in one short-term trial consisting of two balance studies hens fed the phytase showed a higher phosphorus retention compared to the control at 100 FTU/kg feed. In one long-term trial, hens fed 200 FTU/kg feed showed improvements on the performance of the hens. Therefore, there are no sufficient data to conclude on the efficacy of the additive on laying hens.

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Technical dossier FAD-2014-0044/Supplementary information February 2017/Annex 65.
3.3.4. Efficacy for weaned piglets

One short-term trial and three long-term trials were evaluated.

3.3.4.1. Short-term trial

A balance trial was performed with 48 castrated male weaned piglets ([Danish Landrace × Large White (F1)] × Duroc, 29 days old, body weight 9.4 kg) which were allocated to six dietary treatments representing eight piglets (replicates) per treatment. A basal diet based on maize barley and soya bean meal was either not supplemented (control, total phosphorus content 0.39–0.43%; calcium content 0.42–0.43%) or supplemented with Natuphos® E 5000 to provide, 125, 250, 375 or 500 FTU/kg feed. Enzyme activities were confirmed by analysis. A positive control diet was also considered (total phosphorus content 0.57/0.63%; calcium content 0.67/0.68%). All experimental diets contained titanium dioxide as an external marker. The balance study consisted of 30 days of adaptation to the diet and 5 days of collection of faeces and urine (separated). Test diets were fed in pelleted form and restrictively at 2.8× metabolisable energy for maintenance. Body weight and feed intake were measured throughout the study. At the end of the balance trial, all piglets were killed to collect digesta samples from ileum and metacarpal bones (os metacarpale III) from the left front leg. Ileal, faecal and urine samples were analysed to study phosphorus utilisation. Digestibility and utilisation of calcium and phosphorus were calculated. Bone strength was measured and analysed for total ash, calcium and phosphorus content. An ANOVA was done with the data and group means were compared using Tukey test.

No pig died during the experiment and there were no significant differences between the treatments regarding the feed intake or the body weight. Results on the apparent ileal phosphorus digestibility, phosphorus retention and phosphorus bone content are presented in Table 10. The addition of the phytase from 125 FTU/kg feed resulted in a significantly higher ileal digestibility of phosphorus and a higher retention of phosphorus compared to the control.

Table 10: Effect of Natuphos® E on apparent ileal phosphorus digestibility, phosphorus retention and phosphorus bone content in weaned piglets

| Group FTU/kg feed | Phosphorus (%) | Ileal digestibility | Retention | Bone content |
|-------------------|----------------|---------------------|-----------|--------------|
|                   |                |                     |           |              |
| Control           |                | 29.9c               | 25.1e     | 6.8          |
| 125               |                | 39.4b               | 36.0d     | 7.4          |
| 250               |                | 39.7b               | 44.1bc    | 7.3          |
| 375               |                | 41.1b               | 52.5d     | 7.3          |
| 500               |                | 49.3a               | 50.8ab    | 7.3          |
| Positive control  |                | 39.6b               | 41.0cd    | 7.6          |

1: Reported as percent of fat-free dry matter.

Table 9: Effect of Natuphos® E on the phosphorus utilisation in laying hens in the first long-term trial

| Group FTU/kg feed | P retention (%) | Tibia bone (%) | Ash | P |
|-------------------|-----------------|----------------|-----|---|
|                   |                 |                |     |   |
| Control           | 30.7bc          | 52.9a          | 9.1ab|   |
| 100               | 28.3c           | 52.1b          | 8.7bc|   |
| 200               | 32.5abc         | 49.6b          | 8.2c |   |
| 300               | 34.4abc         | 51.5b          | 8.8bc|   |
| 500               | 37.7a           | 51.3b          | 8.7bc|   |
| Positive control  | 29.5bc          | 57.0a          | 9.6a |   |

1: Values reported as percent of fat free dry matter.

a,b,c: Values within one trial and within one column with different superscripts are significantly different (p < 0.05).
3.3.4.2. Long-term trials

Three long-term trials were submitted; these trials included performance data and also measurements on the phosphorus digestibility and bone composition. The first one was the tolerance trial, and the trial design was described in Section 3.2.2.4.85

In the second long-term trial,86 a total of 144 weaned male and female piglets ([Duroc × Landrace] × Pietrain, 26 days old) were penned in groups of three piglets and distributed to six dietary treatments according to body weight (representing eight replicate pens per treatment). Basal diets, prestarter and starter, based on maize and soybean meal (total phosphorus content 0.42% and 0.40%; calcium content 0.55% and 0.52%) were either not supplemented (control) or supplemented with Natuphos® E 10000 L to provide 125, 250, 500 or 1,000 FTU/kg feed. Enzyme activities were confirmed by analysis. A positive control was also considered (total phosphorus content 0.55%; calcium content 0.80–0.79%). Starter diets presented titanium dioxide as an external marker. Diets were offered in pelleted form and on *ad libitum* basis for 42 days (days 1–21 prestarter, days 21–42 starter).

In the third long-term trial,87 a total of 144 castrated male weaned (Goland × Italian Duroc, 31 days old) were penned in groups of four piglets each and distributed to four dietary treatments (representing nine replicate pens per treatment). Basal diets, prestarter and starter, based on maize and soya bean meal (total phosphorus content 0.42% and 0.40%; calcium content 0.55% and 0.52%) were either not supplemented (control) or supplemented with Natuphos® E 5000 to provide 125, 250 or 375 FTU/kg feed. Enzyme activities were confirmed by analysis. All experimental diets contained chromium oxide as an external marker for the digestibility determinations and were offered in mash form for 42 days (day 1–21 prestarter, day 21–42 starter).

In the three trials, health and mortality were monitored, feed intake and body weights were measured and feed to gain ratio were calculated. Samples of faeces were collected on days 18–21 in trial 1, days 39–42 of study in trial 2 or days 38–42 in trial 3. Faecal samples were pooled per pen. At the end of the study, one piglet per pen in trials 1 and 2 or six piglets per group in trial 3 were killed. Samples of digesta contents in ileum (trial 3) and metacarpal bones (Os metacarpale III, all trials) from the left front leg were collected. Ileal and faecal samples were analysed to study the digestibility of phosphorus and bones were analysed for ash content. An ANOVA was done with the data and Tukey test (trials 1 and 2) or Dunnett test (trial 3) was used for the comparison of the group means.

Results of the performance are presented in Table 11 and the data on the digestibility of phosphorus and bone phosphorus content are presented in Table 12.

### Table 11: Effect of Natuphos® E on the performance of weaned piglets

| Trial | Group FTU/kg feed | Daily feed intake (g) | Body weight (kg) | Average daily gain (g/day) | Feed to gain ratio |
|-------|-------------------|-----------------------|-----------------|---------------------------|-------------------|
| 1     | Control           | 558<sup>b</sup>       | 7.4             | 21.2<sup>a</sup>         | 329<sup>b</sup>  | 1.69<sup>a</sup> |
|       | 125               | 598<sup>ab</sup>      | 7.3             | 23.8<sup>a</sup>         | 393<sup>a</sup>  | 1.52<sup>bc</sup> |
|       | 250               | 603<sup>ab</sup>      | 7.3             | 23.1<sup>ab</sup>        | 375<sup>ab</sup> | 1.61<sup>ab</sup> |
|       | 500               | 658<sup>a</sup>       | 7.3             | 25.0<sup>a</sup>         | 422<sup>a</sup>  | 1.56<sup>bc</sup> |
|       | 50,000            | 595<sup>ab</sup>      | 7.3             | 24.4<sup>a</sup>         | 406<sup>a</sup>  | 1.46<sup>c</sup> |
|       | Positive control  | 621<sup>ab</sup>      | 7.3             | 24.1<sup>a</sup>         | 398<sup>a</sup>  | 1.56<sup>bc</sup> |
| 2     | Control           | 503<sup>b</sup>       | 8.2             | 20.9<sup>a</sup>         | 304<sup>b</sup>  | 1.65          |
|       | 125               | 574<sup>ab</sup>      | 8.1             | 23.4<sup>ab</sup>        | 364<sup>a</sup>  | 1.57          |
|       | 250               | 559<sup>ab</sup>      | 8.1             | 23.0<sup>ab</sup>        | 355<sup>ab</sup> | 1.58          |
|       | 500               | 594<sup>a</sup>       | 8.1             | 24.1<sup>a</sup>         | 380<sup>a</sup>  | 1.56          |
|       | 1,000             | 603<sup>a</sup>       | 8.2             | 24.9<sup>a</sup>         | 399<sup>a</sup>  | 1.53          |
|       | Positive control  | 562<sup>ab</sup>      | 8.1             | 23.1<sup>ab</sup>        | 356<sup>ab</sup> | 1.58          |

85 Technical dossier FAD-2014-0044/Section III/Annex IV.2 and Supplementary information February 2017/Annex 31.
86 Technical dossier FAD-2014-0044/Section IV/Annex IV.1 and Supplementary information February 2017/Annex 55.
87 Technical dossier FAD-2014-0044/Section IV/Annex IV.3.
Mortality was low (below 2%) and not treatment related. A higher body weight, average daily weight gain or feed to gain ratio were observed in three trials from the dose of 125 FTU/kg feed in trials 1 and 2 and at the dose of 375 FTU/kg feed in trial 3. The digestibility data and bone analysis showed that groups receiving the phytase had a higher phosphorus digestibility coupled with a higher bone ash content in two trials (trials 1 and 2) from the dose of 250 FTU/kg feed. In trial 3, piglets receiving the phytase at 125 FTU/kg feed showed a significantly higher ash and phosphorus content compared to control but no differences in the digestibility of phosphorus were identified.

Based on the results obtained in the short-term trial and in two long-term trials, trials 1 and 2, the Panel concludes that the additive has a potential to be efficacious in improving the utilisation of phosphorus and the performance of the weaned piglets at 125 FTU/kg feed.

The minimum dose tested is slightly higher than the one that was recommended initially by the applicant. The conclusions on the tolerance were drawn based on the recommended dosage (100 FTU/kg); however, the dosages tested, parameters measured and the results obtained in the tolerance trial allow the Panel to consider that the dose of 125 FTU/kg feed is safe for weaned piglets.

Table 12: Effect of Natuphos® E apparent digestibility of phosphorus and bone mineralisation in weaned piglets

| Trial | Group FTU/kg feed | Daily feed intake (g) | Body weight (kg) | Average daily gain (g/day) | Feed to gain ratio |
|-------|-------------------|----------------------|-----------------|---------------------------|------------------|
|       |                   | Initial | Final |                           |                  |
| 3     | Control           | 602    | 6.9   | 19.3                       | 292              |
|       | 125               | 585    | 7.0   | 19.6                       | 301              |
|       | 250               | 588    | 6.9   | 19.6                       | 301              |
|       | 375               | 586    | 6.9   | 20.4                       | 322*             |

a,b,c: In trials 1 and 2, values within one column for the same trial with different superscripts are significantly different (p < 0.05).

*: In trial 3, values within one column with an asterisk are significantly different from control diet (p < 0.05).

Table 12: Effect of Natuphos® E apparent digestibility of phosphorus and bone mineralisation in weaned piglets

| Trial | Group FTU/kg feed | Digestibility (%) | Bone content (%) |
|-------|-------------------|-------------------|-----------------|
|       |                   | Ileal | Faecal\(^1\) | Ash | P |
| 1     | Control           | –    | 9.8\(^d\) | 30.1\(^d\) | – |
|       | 125               | –    | 21.0\(^c\) | 33.0\(^cd\) | – |
|       | 250               | –    | 33.3\(^b\) | 36.0\(^bc\) | – |
|       | 500               | –    | 42.0\(^b\) | 36.6\(^bc\) | – |
|       | 50,000            | –    | 69.2\(^a\) | 39.0\(^ab\) | – |
|       | Positive control  | –    | 41.2\(^b\) | 42.6\(^a\) | – |
| 2     | Control           | –    | 21.4\(^c\) | 29.1\(^b\) | – |
|       | 125               | –    | 29.6\(^c\) | 31.7\(^ab\) | – |
|       | 250               | –    | 45.2\(^b\) | 32.8\(^a\) | – |
|       | 500               | –    | 58.7\(^a\) | 34.3\(^a\) | – |
|       | 1,000             | –    | 66.4\(^a\) | 34.5\(^a\) | – |
|       | Positive control  | –    | 45.1\(^b\) | 34.2\(^a\) | – |
| 3     | Control           | 42.4 | 47.7/46.7 | 23.5 | 3.64 |
|       | 125               | 47.1 | 49.3/52.5 | 28.3*| 4.41*|
|       | 250               | 47.6 | 51.4/53.7 | 29.0*| 4.40*|
|       | 375               | 50.5 | 57.9/57.2 | 30.0*| 4.51*|

1: In trial 3, the values are for the first (days 17–21) and second period (days 38–42), respectively.
2: Reported as per cent of dry bone in trials 1 and 2 and as per cent of dry matter in trial 3.
a,b,c,d: For trials 1 and 2, values within one column for the same trial with different superscripts are significantly different (p < 0.05).
*: In trial 3, values within one column with an asterisk are significantly different from control diet (p < 0.05).

3.3.5. Efficacy for pigs for fattening

Two short-term trials and four long-term trials in pigs for fattening were evaluated.
3.3.5.1. Short-term trials

The two short-term trials followed the same experimental design. In each study, a total of 24 pigs for fattening ([Danish Landrace × Large White (F1)] × Duroc, 80–90 days old, body weight 32–39 kg) were used. A basal diet based on maize barley and soya bean meal was either not supplemented (control, total phosphorus content 0.31%; calcium content 0.49–0.52%) or supplemented with Natuphos® E 5000 to provide 100, 200 or 300 FTU/kg feed in trial 1 or 100, 300 or 500 FTU/kg feed in trial 2. Enzyme activities were confirmed by analysis. The experiments were conducted in three runs each including 12 pigs per run, it is noted that those involved in the third run had already been involved in the previous runs; however, the diets received were different. In total, data from nine pigs per treatment were available. All experimental diets contained titanium dioxide as an external marker. The balance studies consisted of 9 days of adaptation to the diet and five days of collection of faeces and urine (separated). Test diets were fed in pelleted form and restrictively at 2.8 metabolisable energy for maintenance. Body weight and feed intake were measured throughout the study. Faecal and urine samples collected were analysed to study the retention of phosphorus. An ANOVA was done with the data and group means were compared using Tukey test.

Results of phosphorus retention in trial 1 were 16.2%, 26.7%, 31.0% and 31.6% for control, 100, 200 and 300 FTU/kg feed, respectively, and in trial 2 were 19.5%, 25.0% 28.3% and 34.9% for control, 100, 300 and 500 FTU/kg feed, respectively. Phosphorus utilisation in the groups supplemented with the phytase was significantly higher compared to control from 100 FTU/kg feed in trial 1 and from 300 FTU/kg feed in trial 2.

3.3.5.2. Long-term trials

The first two studies followed a similar experimental design and included the measurements of performance, digestibility and bone mineralisation. A total of 144 (trial 1) or 72 (trial 2) male and female pigs ([Duroc × Landrace] × Pietrain, 70 days old) were penned in groups of three (trial 1) or individually (trial 2), sex separate, and allocated to four (trial 1) or six (trial 2) dietary treatments (representing 12 replicates per treatment). Basal diets, two grower diets and a finisher, based on maize, barley and soya bean meal (total phosphorus content 0.34–0.38%; calcium content 0.45–0.54%) were either not supplemented (control) or supplemented with Natuphos® E 10000 L to provide 100, 200 or 300 FTU/kg feed. In trial 2, a further control diet was considered to study the effect of the phytase in a diet with a lower content of calcium, for which basal diets, two grower and a finisher, based on maize, barley and soybean meal (total phosphorus content 0.35–0.38%; calcium content 0.32–0.50%) were either not supplemented (control low calcium) or supplemented with Natuphos® E 10000 L to provide 300 FTU/kg feed. Enzyme activities were confirmed by analysis. During the first 2 weeks of each phase (days 1-14, 36-49 and 71-84), diets presented titanium dioxide as an external marker. Diets were offered in pelleted form and ad libitum for 105 days. Health status and mortality were monitored throughout the study. Feed intake was measured daily and body weight was measured on days 1, 35, 70 and 105. Feed to gain ratio was calculated. On days 12–14, 47–49 and 82–84, fresh faeces were sampled and pooled per pen to study digestibility of phosphorus and calcium. On day 105 of experiment, 12 pigs per treatment were killed and metacarpal bones (os metacarpale III) from the left front leg were collected. Bones were analysed for ash content. An ANOVA was done with the data and group means were compared with Dunnett test.

Trials 3 and 4 were performance trials done with males and females, respectively. In each study, a total of 72 pigs ([Large White × Landrace] × Pietrain; 70 days of age; 26 kg body weight) were penned in groups of four pigs according to body weight and allocated to two groups, representing nine replicates per group. Basal diets, two grower diets and a finisher, based on maize soya bean meal and rapeseed meal (total phosphorus content 0.36–0.38%; calcium content 0.51–0.53%) were either not supplemented (control) or supplemented with Natuphos® E 5000 G to provide 100 FTU/kg feed. Enzyme activities were confirmed by analysis. Diets were offered ad libitum in pelleted form for 105 days. The pigs were individually weighed at the start of the trial (day 0), at days 34, 69 and at the end of the experiment (day 105). Feed intake for each pen was measured for the same periods and gain to feed ratio was calculated. An ANOVA was performed with the data, pen basis.

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88 Technical dossier FAD-2014-0044/Section IV/Annex IV.5 and Supplementary information February 2017/Annex 14.
89 Technical dossier FAD-2014-0044/Section IV/Annex IV.6 and Supplementary information February 2017/Annex 15.
90 Technical dossier FAD-2014-0044/Section IV/Annex IV.7 and Supplementary information February 2017/Annex 43.
91 Technical dossier FAD-2014-0044/Section IV/Annex IV.8. and Supplementary information February 2017/Annex 44.
92 Technical dossier FAD-2014-0044/Supplementary information February 2017/Annexes 52 and 53.
Results on the performance of the pigs for fattening are presented in Table 13 and the results on the digestibility and bone ash content for trials 1 and 2 are presented in Table 14.

Mortality in the studies was low and not treatment related. Pigs receiving the phytase showed a higher feed intake in three trials, at the dose of 100 FTU/kg in two trials and from 200 FTU/kg in trial 1, and a higher final body weight in the four trials from 200 FTU/kg feed in trials 1 and 2, and at the dose of 100 FTU/kg feed in trials 3 and 4. The Panel notes that trials 3 and 4 were conducted in the same trial site and using the same diets; the only difference was that in trial 3, data in males was reported while in trial 4 data for females was reported. Therefore, the Panel cannot consider trials 3 and 4 as independent.

Table 13: Effect of Natuphos® E on the performance of pigs for fattening

| Study | Group FTU/kg feed | Feed intake (kg/day) | Initial body weight (kg) | Final body weight (kg) | Conversion ratio\(^1\) | Dead/culled pigs (n) |
|-------|------------------|----------------------|--------------------------|------------------------|-----------------------|----------------------|
| 1\(^{10}\) | Control | 1,536 | 27.4 | 85.0 | 0.36 | 0/0 |
| 100 | 1,681 | 27.3 | 90.9 | 0.36 | 1/2 |
| 200 | 1,802* | 27.4 | 99.3* | 0.38 | 2/1 |
| 300 | 1,846* | 27.5 | 98.9* | 0.37 | 0/4 |
| 2\(^{91}\) | Control | 1,944 | 23.6 | 94.9 | 2.92 | 0/0 |
| 100 | 1,984 | 23.6 | 101.9 | 2.74 | 0/0 |
| 200 | 2,055 | 23.6 | 105.4* | 2.70 | 0/0 |
| 300 | 2,040 | 23.6 | 106.1* | 2.65 | 0/0 |
| Control low Ca | 1,827 | 23.6 | 94.9 | 2.76 | 0/1 |
| 300 | 2,015* | 23.6 | 106.8* | 2.61 | 1/0 |
| 3\(^2\) | Control | 1,269\(^b\) | 26.1 | 71.9 | 0.34 | 1/1 |
| 100 FTU/kg | 1,533\(^a\) | 26.0 | 87.7* | 0.38* | 1/0 |
| 4\(^3\) | Control | 1,308\(^b\) | 25.7 | 71.7 | 0.34 | 0/2 |
| 100 FTU/kg | 1,502\(^a\) | 25.8 | 79.9* | 0.34 | 2/0 |

1: Conversion ratio for trials 1, 3 and 4 is gain to feed ratio and for trial 2 is feed to gain ratio.
2: Technical dossier FAD-2014-0044/Supplementary information February 2017/Annex 52.
3: Technical dossier FAD-2014-0044/Supplementary information February 2017/Annex 53.
\(^*:\) Values within one trial and within one column with an asterisk are significantly different from the respective control diet (p < 0.05).

In trials 1 and 2, faecal apparent digestibility of phosphorus was significantly higher in the pigs that received the phytase and this result was coupled with a significantly higher content of ash in bone. The significant effect was observed from 100 FTU/kg feed onwards in trial 2 and from 200 FTU/kg feed in trial 1.

Table 14: Effect of Natuphos® E on apparent faecal phosphorus digestibility and ash bone content in pigs for fattening

| Study | Group       | Phosphorus digestibility (%) | Ash content (%) |
|-------|-------------|------------------------------|-----------------|
|       |             | Days 12–14                   | Days 47–49      | Days 82–84      |
| 1     | Control     | 7.7                          | 20.3            | 26.5            | 38.7 |
| 100   | 20.0*       | 37.0*                        | 37.2*           | 39.6            |
| 200   | 25.2*       | 46.6*                        | 46.0*           | 43.8*           |
| 300   | 36.6*       | 50.2*                        | 52.3*           | 43.9*           |
|       |             | Days 10–12                   | Days 44–46      | Days 78–80      |
| 2     | Control     | 10.6                         | 24.2            | 29.6            | 35.8 |
| 100   | 18.5*       | 32.8                         | 42.7*           | 41.4*           |
| 200   | 28.5*       | 42.5*                        | 41.9*           | 44.0*           |
| 300   | 27.8*       | 32.7                         | 45.7*           | 45.7*           |
| Control low calcium | 0.8  | 24.8                         | 25.4            | 36.1            |
| 300   | 33.5*       | 47.5*                        | 54.9*           | 44.5*           |

\(^*:\) Values within one column with an asterisk are significantly different from the respective control diet (p < 0.05).
In summary, pigs for fattening fed diets with 100 FTU/kg feed showed higher phosphorus retention in one short-term trial, increased phosphorus faecal apparent digestibility coupled with higher ash content in bones in one of the long-term trials and a better growth in another long-term trial. Therefore, the Panel concludes that the additive has a potential to be efficacious to improve the utilisation of phosphorus and the performance of pigs for fattening at 100 FTU/kg feed.

3.3.6. Efficacy for sows

The Panel evaluated five trials in which the effect of the phytase on the phosphorus digestibility was measured in sows, two studies provided data in the lactating period only and three studies provided data in the gestating and lactating periods.

The two studies done in the lactation period followed the same experimental design.93,94 A total of 32 (trial 1) or 40 (trial 2) primiparous Rattlerow Seghers hybrid sows were housed in individual farrowing pens and allocated to four dietary treatments (representing 8 or 10 sows per treatment in trial 1 and in trial 2, respectively). The lactation diets based on maize, barley and soybean meal (total phosphorus content 0.47/0.49%; calcium content 0.59/0.58%, respectively) were either not supplemented (control) or supplemented with Natuphos® E 5000 to provide 100, 250 or 400 FTU/kg feed in trial 1 or 100, 250 or 500 FTU/kg feed in trial 2. Enzyme activities were confirmed by analysis; in trial 2, the diets presenting 100 and 250 FTU/kg feed showed lower values than the intended, 30 and 160 FTU/kg feed, respectively. The feeds presented titanium dioxide as an external marker and were fed to the sows in mash form and ad libitum from day 7 prior to farrowing to day 26 of lactation (33 days of feeding). Sows were weighed at the start and at the end of the experiment and faecal samples were collected on days 22–26 of lactation in order to study the digestibility of phosphorus. Litter performance data were not provided. An ANOVA was done with the data and group means were compared with Tukey test. Results are shown in Table 15.

The third trial is the tolerance trial presented in Section 3.2.2.5.92 In that study, faeces from sows were collected in the gestation (days 85–89) and lactation phase (days 16–20 of lactation). Diets fed to the sows presented an external marker from 9 days before the collection of samples. Faecal samples were analysed to study the digestibility of phosphorus. Litter performance data was reported. An ANOVA was done with the data and group means were compared with Tukey test. Results are presented in Table 15.

The fourth and the fifth trials followed a similar design and studied the faecal apparent phosphorus digestibility in the gestation and lactation phase.95,96 A total of 27 (trial 4) or 30 (trial 5) gestating sows (Duroc x Landrace) were allocated to three (trial 4) or two (trial 5) treatments. Sows were under study from pregnancy confirmation until weaning on day 28 of lactation, total of 105 days. Sows received gestation or lactation diets based on maize and soybean meal (total phosphorus content 0.40% and 0.44% for gestation and lactation, respectively; calcium content 0.60% and 0.66%) that were either not supplemented (control) or supplemented with Natuphos® E to provide 100 or 250 FTU/kg feed in trial 4 or 100 FTU/kg feed in trial 5. Enzyme activities were confirmed by analysis. The gestation diet was offered in pelleted form and sows were restrictively fed. Then, after the lactation diet was offered in pelleted form and on ad libitum basis (following an increasing controlled curve). Health status and mortality of sows and piglets were monitored throughout the study. Sow’s weight was measured at the start of the experiment, last week of gestation, after farrowing and at weaning. Feed intake during lactation was recorded daily. At farrowing, number of piglets born and their body weight were recorded. Litter weight was measured on days 21 and 28 of lactation. Cross-fostering of piglets was performed during the first 3 days after farrowing. Apparent faecal digestibility was measured on days 85–88 of gestation and during lactation on days 18–22. Feed administered between days 71–88 of gestation and days 1–22 of lactation contained titanium dioxide as an external marker. An ANOVA was done with the data and group means were compared with Tukey test. Results are presented in Table 15.

93 Technical dossier FAD-2014-0044/Section IV/Annex IV.10 and supplementary information February 2017/Annex 16
94 Technical dossier FAD-2014-0044/Supplementary information February 2017/annex 60
95 Technical dossier FAD-2014-0044/Section IV/Annex IV.9 and Supplementary information February 2017/Annex 70
96 Technical dossier FAD-2014-0044/Supplementary information February 2017/annex 17
In the short-term trials, no sow died during the experiment; in trial 1, one sow was removed from the trial due to health problems. During lactation, there were no significant treatment-related effects on body weight of sows or body weight change. Apparent faecal digestibility of phosphorus of the sows was higher compared to control in sows receiving the phytase from 100 FTU/kg feed in trial 1 and from 250 FTU/kg feed in trial 2. However, these trials did not include measurements on the litter performance during the lactation.

In the other trials (trials 3–5), sows in the gestation phase fed the phytase showed a higher apparent faecal digestibility of phosphorus at 2,500 FTU/kg feed in trial 3 and from 100 FTU/kg feed in trial 4. During the lactation phase, sows fed the phytase showed a higher apparent faecal digestibility of phosphorus from 250 FTU/kg feed in trial 3, at 250 FTU/kg feed in trial 4 and at 100 FTU/kg feed in trial 5. Performance parameters of the litter showed no differences between the treatments.

Improvements on the apparent faecal digestibility of phosphorus were seen in three trials at 100 FTU/kg feed (one in gestation and two in the lactation phase). The Panel acknowledges that in one of the studies in the lactation phase no data on the litter performance was reported. These data would be required in order to check that no differences in the growth of the litters happened concurrently with improvements on the digestibility of phosphorus. The results from other studies in the lactation phase showed no negative impact of feeding the phytase on the performance of the litters. Consequently, the Panel considers that the results form that trial on the apparent faecal digestibility of phosphorus can be considered in the assessment and therefore concludes that additive has a potential to improve phosphorus availability in sows at the dose of 100 FTU/kg feed.

### 3.3.6.1. Conclusions on efficacy for the target species

The Panel concludes that Natuphos® E has a potential to be efficacious in improving the performance of the animals and/or the utilisation of phosphorus in chickens for fattening at a minimum dose of 750 FTU/kg feed, in turkeys for fattening and weaned piglets at a minimum dose of 125 FTU/kg feed, and in pigs for fattening and sows at a minimum dose of 100 FTU/kg feed. However, there is insufficient data in laying hens to conclude on the efficacy of the additive. The conclusions on the chickens and turkeys for fattening can be extended to chickens and turkeys reared for laying/breeding at the corresponding dose.

The mode of action of the phytase is well known and can be considered to be similar in all poultry/avian species. Therefore, the conclusions drawn in turkeys for fattening can be extrapolated to all minor poultry species and other avian species up to the point of lay (efficacious dose at 125 FTU/kg feed). Similarly, the conclusions drawn in pigs for fattening (100 FTU/kg feed) and sows (100 FTU/kg feed) can be extrapolated to minor porcine species for growing and reproduction, respectively. No conclusion can be drawn on the minor poultry and other avian species for laying.

The Panel notes that the efficacious dose in chickens for fattening (750 FTU/kg feed) and weaned piglets (125 FTU/kg feed) are higher than the minimum recommended doses. The conclusions on the tolerance were drawn based on the recommended dosages. However, the respective tolerance trials allow the Panel to consider that the efficacious dosages are safe for chickens for fattening and weaned piglets.

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**Table 15:** Effect of Natuphos® E on the apparent faecal phosphorus digestibility (%) in sows

| Trial | Gestation | Lactation | Gestation | Lactation |
|-------|-----------|-----------|-----------|-----------|
|       | Control   | 100       | 250       | 400       | 500       | 2,500    | Control | 100 | 250 | 400 | 500 | 2,500 |
| 1     | –         | –         | –         | –         | –         | 32.9b    | –       | –   | –   | –   | –   | –     |
| 2     | –         | –         | –         | –         | –         | 36.0c    | –       | –   | –   | –   | –   | –     |
| 3     | 32.4b     | 32.6b     | 32.3b     | –         | –         | 40.7a    | 31.9c   | 31.1c| 41.2b| –   | –   | 48.3a |
| 4     | 15.3b     | 25.2a     | 32.7a     | –         | –         | 17.5b    | 23.6b   | 36.3a| –   | –   | –   | –     |
| 5     | 18.5      | 20.0      | –         | –         | –         | 30.5b    | 39.1a   | –   | –   | –   | –   | –     |

a,b,c: Values within one row for the same trial and reproductive phase with different superscripts 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 Regulation\(^\text{97}\) and Good Manufacturing Practice.

4. Conclusions

The additive does not give rise to safety concerns with regard to the genetic modification of the production strain. The production strain and its recombinant DNA were not detected in the fermentation product used to formulate the additive.

The tolerance trials showed that chickens and turkeys for fattening tolerated 400-fold the recommended dose, laying hens 250-fold, weaned piglets 500-fold and sows 25-fold. Therefore, the FEEDAP Panel concludes that the additive is safe at the respective recommended dosages. The conclusions in chickens and turkeys for fattening can be extended to chickens reared for laying and to turkeys reared for breeding at the corresponding dose and that reached in piglets can be extended to pigs for fattening. The conclusions can also be extrapolated to minor poultry species and other avian species and to minor porcine species at the corresponding dose.

The use of the product as a feed additive does not give rise to concerns for consumers. The additive Natuphos\(^\text{®} E\) is not considered to be toxic by inhalation or irritant for skin or eye. However, it should be regarded as a dermal sensitiser and a potential respiratory sensitiser.

The use of Natuphos\(^\text{®} E\) as a feed additive poses no risks to the environment. The additive has the potential to be efficacious in improving the performance and/or the phosphorus utilisation in chickens for fattening at 750 FTU/kg feed, in turkeys for fattening and weaned piglets at 125 FTU/kg feed and in pigs for fattening and in sows at 100 FTU/kg feed. The conclusions on the chickens and turkeys for fattening can be extended to chickens and turkeys reared for laying/breeding at the corresponding dose. The conclusions drawn in turkeys for fattening can be extrapolated to all minor poultry species and other avian species for growing or up to the point of lay. Similarly, the conclusions drawn in pigs for fattening and sows can be extrapolated to minor porcine species for growing and reproduction, respectively. There is insufficient data to conclude on the efficacy of the additive in laying hens and on minor poultry and other avian species for laying.

5. Recommendations

The microbial contamination of the batches analysed of Natuphos\(^\text{®} E\) 5000 raises concerns for the safety of the product. Quality control measures should be put in place to ensure that microbial contamination of the final product is minimised.

Documentation provided to EFSA

1) FAD-2014-0044 Natuphos\(^\text{®} E\) for pig and avian species. December 2014. Submitted by BASF SE.
2) FAD-2014-0044 Natuphos\(^\text{®} E\) for pig and avian species. Supplementary information. August 2015. Submitted by BASF SE.
3) FAD-2014-0044 Natuphos\(^\text{®} E\) for pig and avian species. Supplementary information. February 2017. Submitted by BASF SE.
4) FAD-2014-0044 Natuphos\(^\text{®} E\) for pig and avian species. Supplementary information. September 2017. Submitted by BASF SE.
5) FAD-2015-0040 Natuphos\(^\text{®} E\) for pig and avian species. October 2015. Submitted by BASF SE.
6) FAD-2015-0040 Natuphos\(^\text{®} E\) for pig and avian species. Supplementary information. February 2017. Submitted by BASF SE.
7) FAD-2015-0040 Natuphos\(^\text{®} E\) for pig and avian species. Supplementary information. September 2017. Submitted by BASF SE.
8) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Natuphos\(^\text{®} E\).
9) Comments from Member States.

\(^{97}\) Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.
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EFSA (European Food Safety Authority), 2008b. Technical Guidance: microbial studies. EFSA Journal 2008; 6(10):836, 3 pp. https://doi.org/10.2903/j.efsa.2008.836

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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. https://doi.org/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. https://doi.org/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. https://doi.org/10.2903/j.efsa.2012.2539

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Abbreviations

ANOVA analysis of variance
bw body weight
CFU colony forming unit
CV coefficient of variation
EMS ethyl methane sulfonate
EURL European Union Reference Laboratory
FEEDAP EFSA Panel on Additives and Products or Substances used in Animal Feed
IgE immunoglobulin E
i.p. intraperitoneal
ITS internal transcribed spacer
OECD Organisation for Economic Co-operation and Development
PCB polychlorinated biphenyl
PCR polymerase chain reaction
SS signal sequence

Natuphos® E for poultry and pigs
Appendix A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Natuphos® E

FAD-2014-0044

In the current application, authorisation is sought under article 4(1) of the Regulation (EC) No 1831/2003 for Natuphos® E under the category/functional groups 4 (a and c) ‘zootechnical additives’/‘digestibility enhancers’ and ‘substances which favourable affect the environment’. Specifically, authorisation is sought for the use of the feed additive for all pigs and all avian species.

According to the Applicant, 6-phytase is the active agent of Natuphos® E. The Applicant expresses the phytase enzymatic activity in FTU/g units, where ‘one FTU is the amount of enzyme which releases one micromole of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37°C’.

The product is intended to be marketed as powder and liquid formulations having a guaranteed minimum phytase activity ranging from 5,000 to 25,000 FTU/g.98 Natuphos® E is intended to be included through premixtures to obtain a minimum activity of 100 125, 200 or 250 FTU/kg feedingstuffs, depending on the target species.

For the quantification of phytase activity in feedingstuffs, the Applicant submitted the ring-trial validated colorimetric EN ISO 30024 standard method. Furthermore, the Applicant applied (i) the ISO standard with minor experimental modifications to the analysis of the feed additive (Natuphos® E) and (ii) the ring-trial validated colorimetric method (VDLUFA 27.1.3) for the quantification of the phytase activity in premixtures and obtained similar method performance characteristics. Based on the performance characteristics provided, the EURL recommends for official control the colorimetric methods mentioned above for the quantification of phytase activity in the feed additive, premixtures and feedingstuffs.

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

FAD-2015-0040

In the current application, authorisation is sought under article 4(1) of the Regulation (EC) No 1831/2003 for Natuphos® E under the category/functional groups 4 (a and c) ‘zootechnical additives’/‘digestibility enhancers’ and ‘substances which favourable affect the environment’. Specifically, authorisation is sought for the use of the feed additive for all pigs and all avian species.

According to the Applicant, 6-phytase is the active agent of Natuphos® E. The Applicant expresses the phytase enzymatic activity in FTU/g units, where ‘one FTU is the amount of enzyme which releases one micromole of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37°C’.

The product is intended to be marketed as granulate formulations having a guaranteed minimum phytase activity of 5,000 FTU/g (Natuphos® E 5000 G) and of 10,000 FTU/g (Natuphos® E 10000 G). Natuphos® E is intended to be included into feedingstuffs directly and/or through premixtures to obtain a minimum activity ranging from 100 to 250 FTU/kg feedingstuffs, depending on the target species. For the quantification of phytase activity in feedingstuffs, the Applicant submitted the ring-trial validated colorimetric EN ISO 30024 standard method. Furthermore, the Applicant applied (i) the ISO standard with minor experimental modifications to the analysis of the feed additive (Natuphos® E) and (ii) the ring-trial validated colorimetric method (VDLUFA 27.1.3) for the quantification of the phytase activity in premixtures and obtained similar method performance characteristics. Based on the performance characteristics provided, the EURL recommends for official control the colorimetric methods mentioned above for the quantification of phytase activity in the feed additive, premixtures and feedingstuffs.

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

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98 EFSA notes that the formulation containing 25,000 FTU/g was withdrawn from the assessment.