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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of Axtra® PHY 15 000 L (6-phytase) as a feed additive for poultry and porcine species[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The additive Axtra® PHY 15 000 L is a liquid preparation of 6-phytase produced by a genetically modified strain of *Trichoderma reesei*. This product is intended for use as a zootechnical additive, functional group of digestibility enhancers, in feed for poultry and porcine species. The Panel on Additives and Products or Substances used in Animal Feed concluded that Axtra® PHY 15 000 L does not give rise to safety concerns with regard to the genetic modification of the production strain. Neither the production strain nor its recombinant DNA was detected in the additive. The Panel concluded that the additive is safe for chickens and turkeys for fattening, laying hens, weaned piglets and sows at the maximum recommended dose (2 000 phytase units (FTU)/kg feed) and considered that this conclusion can be extended to other poultry categories/species and porcine categories/species at the same maximum dose. The Panel concluded that the use of Axtra® PHY 15 000 L as a feed additive gives rise to no concerns for consumers. This additive is not considered a dermal sensitiser but should be considered an irritant. Owing to the proteinaceous nature of its active substance, the additive is considered a potential respiratory sensitiser. The Panel concluded that the use of Axtra® PHY 15 000 L as a feed additive poses no risks to the environment. The efficacy studies submitted showed that the phytase present in Axtra®PHY 15 000 L is efficacious in chickens and turkeys for fattening, laying hens, piglets, pigs for fattening and sows at the dose of 250 FTU/kg feed. This conclusion can be extended to chickens reared for laying, turkeys reared for breeding, and to turkeys for breeding purposes, and extrapolated to minor poultry species for fattening and minor porcine species, at the same dose.

KEY WORDS

zootechnical additives, digestibility enhancers, 6-phytase, poultry, pigs, efficacy and safety

[1] On request from the European Commission, Question No EFSA-Q-2013-00997, adopted on 22 October 2015.

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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 Axtra® PHY 15 000 L (6-phytase) as a feed additive for poultry and pig species.

The additive Axtra® PHY 15 000 L is a liquid preparation of 6-phytase produced by a genetically modified strain of *Trichoderma reesei*. This product has not been previously authorised in the European Union and is intended for use as a zootechnical additive, functional group of digestibility enhancers, in feed for chickens for fattening or reared for laying, turkeys for fattening, reared for breeding or for breeding purposes, laying hens, minor poultry species, piglets (weaned), pigs for fattening, sows for reproduction, and minor porcine species.

The Panel concluded that Axtra® PHY 15 000 L does not give rise to safety concerns with regard to the genetic modification of the production strain. Neither the production strain nor its recombinant DNA was detected in the final product obtained from the production strain.

Based on the tolerance trials provided, in which a 100-fold the maximum recommended dose was tolerated well by the animals, the Panel concluded that the additive is safe for chickens and turkeys for fattening, laying hens, weaned piglets and sows at the maximum recommended dose (2 000 phytase units (FTU)/kg). The Panel extended this conclusion to chickens reared for laying, to turkeys reared for breeding purposes or for breeding, and to pigs for fattening. The conclusion was also extrapolated to minor poultry and porcine species at the same maximum dose.

The results obtained in genotoxicity and mutagenicity tests and in a sub-chronic oral toxicity study permitted the Panel to conclude that the use of Axtra® PHY 15 000 L as feed additive gives rise to no concerns for consumers.

The additive is not considered a dermal sensitiser but should be considered an irritant to eyes, skin and the respiratory tract. Owing to the proteinaceous nature of the active substance, the additive is considered a potential respiratory sensitiser.

The Panel concluded that the use of Axtra®PHY 15 000 L as a feed additive poses no risks to the environment.

The use of 6-phytase in feed should improve the assimilation of phosphorus and other minerals. The efficacy studies submitted showed that the phytase present in Axtra® PHY 15 000 L is efficacious in chickens and turkeys for fattening, piglets, pigs for fattening and sows at the dose of 250 FTU/kg feed. For laying hens, two studies showed a significant effect at 250 FTU/kg and one at 300 FTU/kg (the lowest dose tested in the study). Considering that for all other species/categories, efficacy was demonstrated at 250 FTU/kg and that the difference between 250 and 300 FTU/kg is small, the Panel concluded that, for laying hens, the effective dose is also 250 FTU/kg. These conclusions were extended to chickens reared for laying, to turkeys reared for breeding and to turkeys for breeding purposes, and were extrapolated to minor poultry species for fattening and minor porcine species at the same dose.

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BACKGROUND

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

The European Commission received a request from Danisco UK Ltd[5] for authorisation of the product Axtra® PHY 15 000 L, 6-phytase, when used as a feed additive for chickens for fattening or reared for laying, turkeys for fattening, reared for breeding of for breeding purposes, laying hens, minor poultry species, piglets (weaned), pigs for fattening, sows for reproduction and minor porcine species (category: zootechnical additive; functional group: digestibility enhancers) under the conditions mentioned in Table 1.

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 dossier in support of this application.[6] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 11 April 2014.

The additive Axtra® PHY 15000 L is a preparation of 6-phytase produced by a genetically modified strain of *Trichoderma reesei* that has not been previously authorised in the European Union.

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 the efficacy of the product Axtra® Phy 15 000 L (6-phytase), when used under the conditions described in Table 1.

[4] 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.
[5] Danisco UK Ltd, PO Box 777, SN8 1XN, Marlborough, Wiltshire, UK.
[6] EFSA Dossier reference: FAD-2013-0049.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	15000 FTU/g 6-phytase			
Registration number/EC No/No (if appropriate)	4axxx			
Category(ies) of additive	Zootechnical additive			
Functional group(s) of additive	Digestibility enhancer			
Description				
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)	
Preparation of 6-phytase (EC 3.1.3.26) produced by Trichoderma reesei	Minimum 6-phytase activity: 15000 FTU/g	Compliant EU feed hygiene law	Colorimetric method	
Trade name (if appropriate)	Axtra® PHY 15000 L			
Name of the holder of authorisation (if appropriate)	Danisco (UK) Ltd. (trading as Danisco Animal Nutrition)			
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		Units of activity/kg of complete feedingstuffs		
Chickens for fattening	To slaughter age & weight	250 FTU/kg	2000 FTU/kg	Not applicable
Chickens reared for laying	24 weeks (point of lay)			
Laying hens	No maximum age			
Turkeys for fattening	To slaughter age & weight			
Turkeys for breeding purposes	No maximum age			
Turkeys reared for breeding	30 weeks (point of lay)			
Weaned piglets	120 days			
Sows for reproduction	No maximum age			
Pigs for fattening	To slaughter age & weight			
Minor porcine species	No maximum age			

Minor poultry species	No maximum age			
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use (if appropriate)	Post-pellet application			
Specific conditions or restrictions for handling (if appropriate)	R42, potential respiratory sensitizer. S22, do not breathe dust. Inhalation of enzyme aerosols or dust may cause allergic reactions in sensitized individuals. For user safety: Avoid direct contact and use in well-ventilated area. Breathing protection, safety glasses, and protective gloves/clothing are recommended			
Post-market monitoring (if appropriate)	In compliance with EU feed hygiene legislation, traceability, HACCP, formal product/service complaints procedure, and product recall capability			
Specific conditions for use in complementary feedingstuffs (if appropriate)	Final complete feed should contain 0.0167-0.133 kg Axtra® PHY 15000 L per tonne feed (minimum 250 FTU 6-phytase/kg feed & maximum 2000 FTU 6-phytase/kg feed).			
Maximum Residue Limit (MRL)				
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues	
Not applicable	Not applicable	Not applicable	Not applicable	

ASSESSMENT

1. Introduction

The additive Axtra® PHY 15 000 L is a liquid preparation of 6-phytase (phytase; EC 3.1.3.26) produced by a genetically modified strain of *Trichoderma reesei*. This product has not previously been authorised in the European Union and is intended for use as a zootechnical additive, functional group of digestibility enhancers, in feed for chickens for fattening or reared for laying, turkeys for fattening, reared for breeding or for breeding purposes, laying hens, minor poultry species, piglets (weaned), pigs for fattening, sows for reproduction, and minor porcine species. The use of 6-phytase in feed should improve the assimilation of phosphorus and other minerals.

2. Characterisation[7]

2.1. Characterisation of the production organism

The 6-phytase present in the additive under assessment is produced by fermentation of a genetically modified strain of *T. reesei*. The technical dossier contains detailed and sufficient information on the recipient microorganism, including aspects on the safety of the strain lineage, the origin and function of the different genetic elements introduced in the production strain, the genetic modification process and the genetic and phenotypic traits introduced.

2.2. Manufacturing process

The product is obtained by a multi-step process consisting in fermentation, concentration and purification steps. The resulting fermentation product is mixed and the final formulation is prepared with the addition of carriers and stabilisers.

2.3. Characterisation of the additive

Axtra® PHY 15 000 L is a light brown liquid preparation with phytase as the main activity, which ensures a guaranteed minimum activity of 15 000 phytase units (FTU)[8]/g. Other activities present are xylanase, beta-glucanase and alpha-amylase activities. The batch-to-batch variation was studied in five batches and the mean value was 23 332 FTU/g, ranging from 21 800 to 24 500 FTU/g (coefficient of variation (CV) of 4.3 %).[9] The additive contains the liquid fermentation product, sorbitol, sodium chloride, potassium sorbate, sodium benzoate and water. The additive has a specific gravity of 1.18–1.20 kg/L (at 20 °C), a pH of 5.3–5.5 and a viscosity of 5.61–6.08 cP (measured at 25 °C).[10]

The additive was analysed for microbiological contaminants (eight batches),[11] including total viable counts (< 78 colony-forming units (CFU)/mL), coliforms (< 1 CFU/mL), *Escherichia coli* and *Salmonella* spp. (not detected in 25 g). Three batches were investigated for their content of lead (< 5 mg/kg), cadmium and mercury (< 0.5 mg/kg), and arsenic (< 3 mg/kg). The levels of mycotoxins, including aflatoxins (< 5 µg/kg), ochratoxin (< 10 µg/kg), zearalenone (< 50 µg/kg), T2-toxin (< 25 µg/kg) and vomitoxin (< 0.5 µg/kg) were also determined in the same batches.[12] Antibacterial activity was reported to be absent in three batches of the additive.[13] The production strain was not detected in three batches of the additive. No recombinant DNA was detected in three batches of the final product tested by polymerase chain reaction.

[7] This section has been edited following the confidentiality claims made by the applicant.
[8] One FTU is the amount of enzyme that releases 1 µmol of inorganic orthophosphate from a sodium phytate substrate per minute at pH 5.5 and 37 °C.
[9] Technical dossier/Section II/Annex II.2.
[10] Technical dossier/Section II/Annexes II.4, II.6 and II.9.
[11] Technical dossier/Section II/Annexes II.2, II.4 and II.6.
[12] Technical dossier/Section II/Annex II.4 and Supplementary information April 2015.
[13] Technical dossier/Section II/Annex II.4 and Supplementary information July 2015.

2.4. Stability and homogeneity

The shelf lives of three batches of Axtra® PHY 15 000 L were studied at 25 and 40 °C.[14] The samples were kept in closed plastic containers for up to 12 months. The initial mean phytase content of the batches was 23 122 FTU/g and the mean recovery of phytase activity after 12 months was 78 % at 25 °C and 71 % at 40 °C.

The additive is intended to be applied by spraying onto pelleted feed. Three batches of the additive were sprayed onto a pelleted complete feed, based on maize and soya bean meal and containing a mineral and vitamin premixture, to provide 450 FTU/kg feed.[15] Samples were kept in plastic containers at 25 or 40 °C for three months. Recovery values after three months were no different from the initial enzyme activity. In order to study the capacity of the additive to homogeneously distribute in feed, 10 sub-samples of each feed were analysed. The CV of the enzyme activity ranged from 5.8 to 6.7 %.

2.5. Conditions of use

The additive is to be used in feed for chickens for fattening or reared for laying, turkeys for fattening or reared for breeding, laying hens, turkeys for breeding purposes and minor poultry species, weaned piglets, pigs for fattening, sows for reproduction, and minor porcine species at a minimum dose of 250 FTU/kg. The maximum recommended dose is 2 000 FTU/kg feed for all species/categories.

2.6. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL report can be found in the Annex.

3. Safety

3.1. Safety aspects of the genetic modification[16]

The recipient organism is considered safe. The introduced sequences give rise to no safety concerns. The product Axtra® PHY 15 000 L does not give rise to any safety concerns with regard to the genetic modification of the production strain.

3.2. Safety for the target species

The test materials used in the tolerance studies were not the final product; for the tolerance group, a concentrated intermediate product (6-phytase concentrate, sodium benzoate and potassium sorbate) was used and, for the use level, a diluted version (three-fold diluted) of the final additive was used. The Panel considers that these test materials were adequate and will be referred in the studies below as Axtra® PHY phytase.

3.2.1. Chickens for fattening

A total of 1 600 one-day-old chickens (Ross 308, male and female) were distributed in groups of 40 birds, separated by sex, and allocated to one of four dietary treatments, resulting in 10 replicates per treatment.[17] Starter and grower basal diets, based on wheat and soya bean meal and with a total phosphorus content of 0.51 % (starter) or 0.42 % (grower), were either not supplemented (negative control group) or supplemented with the Axtra® PHY phytase to provide 2 000 FTU/kg feed (1× group) or 200 000 FTU/kg feed (100× group) (confirmed by analysis). A positive control diet with a total phosphorus content of 0.57 % (starter) or 0.52 % (grower) was also considered. Diets were offered *ad libitum* in pelleted form for 42 days. The feed intake, body weight and mortality were

[14] Technical dossier/Section II/Annex II.21.
[15] Technical dossier/Section II/Annex II.22.
[16] This section has been edited following the confidentiality claims made by the applicant.
[17] Technical dossier/Section III/Annex III.1 and Supplementary information April 2015/Annex III.1.1.

recorded and the feed to gain ratio was calculated. Analysis of variance (ANOVA) was performed with the data including the effects of the dietary treatment, sex, and their interaction. The pen was the experimental unit. The mean values of the groups were compared with the least significant difference (LSD) test. Differences were considered significant at a level of at least $P < 0.05$.

Mortality was within normal limits and was not treatment related (4.7, 5.4, 5.5 and 4.6 % for the negative control, 1×, 100× and positive control groups, respectively). Total feed intake of the birds were 4.9, 5.0, 5.0 and 5.0 kg feed/bird for the negative control, 1×, 100× and positive control groups, respectively, and were not affected by the dietary treatments. Final body weight values were 2.70, 2.83, 2.88 and 2.82 kg for the negative control, 1×, 100× and positive control groups, respectively. The corresponding figures for the feed to gain ratio were 1.83, 1.81, 1.78 and 1.82, respectively. The supplementation of the diet with phytase at either dose resulted in a significantly higher mean body weight than the negative control diet; the 100× group had a significantly better feed to gain ratio. Feeding the birds with the phytase up to 100-fold the maximum dose did not have any negative effects on the performance parameters. Therefore, the FEEDAP Panel concludes that the product is safe for chickens for fattening at the maximum recommended dose.

3.2.2. Safety for turkeys for fattening

A total of 384 one-day-old female turkeys (BUT 9) were distributed in groups of 12 birds and allocated to one of four dietary treatments, resulting in eight replicates per treatment.[18] Starter and grower basal diets, based on maize and soya bean meal and with a total phosphorus content of 0.62 % (starter) or 0.53 % (grower), were either not supplemented (negative control group) or supplemented with Axtra® PHY phytase to 2 000 FTU/kg feed (1× group) or 200 000 FTU/kg feed (100× group) (confirmed by analysis). A positive control diet with a total phosphorus content of 0.79 % (starter) or 0.77 % (grower) was also considered. Diets were offered *ad libitum* in pelleted form for 42 days. The feed intake, body weight and mortality were recorded and the feed to gain ratio was calculated. An ANOVA was performed with the data considering the pen as the experimental unit. Mean values of the groups were compared with the Fisher T-test. Differences were considered significant at a level of at least $P < 0.05$.

One animal died (1× group) and another one was culled (100× group). Total feed intakes were 2.45, 2.95, 2.95 and 2.73 kg feed/bird for the negative control, 1×, 100× and positive control groups, respectively; final body weight values were 1.58, 1.92, 1.92 and 1.72 kg and feed to gain ratio was 1.57, 1.53, 1.55 and 1.58 for the negative control, 1×, 100× and positive control groups, respectively. The supplementation of the diet with Axtra® PHY phytase resulted in a significantly higher feed intake and body weight, and better feed to gain ratio than the negative control diet. Feeding the birds with the phytase up to 100-fold the maximum dose, did not have negative effects on the performance parameters. Therefore, the FEEDAP Panel concludes that the product is safe for turkeys for fattening at the maximum recommended dose.

3.2.3. Safety for laying hens

A total of 400 21-week-old Isa brown hens were caged in groups of 10 hens and allocated to four dietary treatments, resulting in 10 replicates per treatment.[19] A basal diet, based on maize and soya bean meal with a total phosphorus content of 0.44 %, was either not supplemented (negative control group) or supplemented with Axtra® PHY phytase to provide 2 000 FTU/kg feed (1× group) or 200 000 FTU/kg feed (100× group) (confirmed by analysis). A positive control diet with a total phosphorus content of 0.60 % content was also considered. Feed was offered in mash form for 56 days. Body weight of the hens was measured at the beginning and at the end of the study. Health status, feed intake and laying performances were monitored/recorded throughout the study. Quality parameters, including yolk colour, albumen weight, egg shell thickness and shell index (% of egg weight) were measured in all eggs laid in a 24-hour period on weeks 4 and 8. At the end of the trial,

[18] Technical dossier/Section III/Annex III.3.
[19] Technical dossier/Supplementary information April 2015/Annex III.14.

blood samples from 10 animals from each group were collected and haematological and biochemical parameters were determined.[20] An ANOVA was performed with the data considering the cage as the experimental unit. Mean values of the groups were compared with the Fisher T-test. Differences were considered significant at a level of at least $P < 0.05$.

Seven hens died, six from the negative control group and one from the 100× group. Daily feed intakes were 111, 110, 109 and 113 g for the negative control, 1×, 100× and positive control groups, respectively, laying intensities were 90.4, 91.8, 89.9 and 91.0 %, egg mass production values were 28.0, 29.7, 28.7 and 29.6 kg/cage, feed to egg mass ratios were 2.07, 2.04, 2.08 and 2.12, and egg weights were 59.8, 59.0, 58.6 and 58.9 g/egg and were not affected by the addition of phytase. The body weight of the hens was not affected by the treatments. Egg quality parameter measurements showed some differences related to the dietary treatments, but these were not dose related. The results of the blood analyses showed a decrease in the number of heterophils in the phytase supplemented groups (16.1×10^3 cells/µL in the non-supplemented vs. 8.4×10^3 and 7.3×10^3 cells/µL in the 1× and 100× groups, respectively); however, values in the phytase-supplemented groups were not different to those in the positive control group (10.8×10^3 cells/µL). Feeding the laying hens with the phytase up to 100-fold the maximum dose, did not have negative effects on the laying performance or egg quality, or on biochemical and haematological blood parameters. Therefore, the FEEDAP Panel concludes that the product is safe for laying hens at the maximum recommended dose.

3.2.4. Safety for weaned piglets

A total of 128 weaned male piglets (Landrace × Large White, 35–39 days old and initial mean body weight of 11.28 kg) were distributed into pens in groups of four piglets each and allocated to four dietary treatments, resulting in eight replicates per treatment.[21] Weaner and starter diets, with a total phosphorus content of 0.48 % (weaner) and 0.41 % (starter), were either not supplemented (negative control group) or supplemented with Axtra® PHY phytase to provide phytase at 2 000 FTU/kg of diet (1× group) and 200 000 FTU/kg of diet (100× group) (confirmed by analysis). A positive control diet with a total phosphorus content of 0.65 % (weaner) or 0.57 % (starter) was also considered. Before the start of the tolerance trial (28–32 days of age), the piglets had a seven-day acclimatisation period in which the animals were given antibiotics via water for five days. Feed and water were available *ad libitum* over an experimental period of 42 days. The weaner diet (pelleted feed) was offered from day 1 until day 14 and the starter diet (pelleted feed) from day 15 until day 42 of the experiment. Body weight and feed intake were recorded at days 0, 14 and 42, and average daily gain, average daily feed intake and feed to gain ratio were calculated. In addition, on day 42 of the experiment, blood samples were obtained from 10 piglets per treatment (randomly selected) for routine blood haematology and biochemistry.[22] An ANOVA was performed with the data considering the pen as the experimental unit. Mean values of the groups were compared with the Tukey test. Differences were considered significant at a level of at least $P < 0.05$.

A total of 13 piglets (four in the negative control group, eight in the 1× group and one in the 100× group) had to be individually treated with short courses of antibiotics for various reasons. Daily feed intakes were 0.98, 1.11, 1.04 and 1.09 kg for the negative control, 1×, 100× and positive control groups, respectively. The final body weight values were 35.8, 40.6, 39.7 and 40.3 kg, daily weight gains were 0.587, 0.698, 0.677 and 0.687 kg, and feed to gain ratios were 1.67, 1.59, 1.54 and 1.59 for

[20] Including: calcium, phosphorus, creatinine, potassium, glucose, urea, bilirubin, lactate, fibrinogen, electrolytes (sodium, potassium chloride and calcium), total protein, albumin, globulin (and ratio of albumin to globulin), gamma-glutamyltransferase (γGT), alkaline phosphatase (AP), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and alanine aminotransferase (ALT), haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), haematocrit, red blood cell count, red blood cell distribution width, white blood cell count (including differential counts) and thrombocytes.

[21] Technical dossier/Supplementary information April 2015/Annex III.15.

[22] Including: fibrinogen, haemoglobin, mean corpuscular haemoglobin, MCHC, MCV, packed cell volume, platelets, red blood cell count and white blood cell counts (including differential counts), total protein, albumin, globulin, albumin to globulin ratio, urea, creatinine, total billirubin, glucose, inorganic phosphorus, chloride, potassium, sodium, calcium, AP, ALT, AST, γGT and LDH activity.

the negative control, 1×, 100× and positive control groups, respectively. Body weights and daily weight gains in the 1× and 100× groups were higher than those in the negative control group, and the feed to gain ratio in the 100× group was better than in the negative control. Compared with the negative control diet, phytase supplementation at either dose increased inorganic phosphorus concentrations and decreased the alkaline phosphatase activity, supplementation at 100-fold increased the alanine aminotransferase (ALT) activity and decreased the mean corpuscular haemoglobin concentration. Since the values for the phytase-supplemented groups were no different from the values for the positive control group, these modifications are considered of no concern. Therefore, the supplementation of the diets with the phytase contained in Axtra® PHY, at levels up to 100-fold the maximum recommended dose, did not have a negative effect on the parameters measured. The FEEDAP Panel concludes that the additive is safe for weaned piglets at the maximum recommended dose.

3.2.5. Safety for pigs for fattening

No specific study was provided to demonstrate the tolerance of pigs for fattening. The FEEDAP Panel considers that the conclusions reached with regard to piglets can be extended to pigs for fattening.

3.2.6. Safety for sows for reproduction

A total of 48 mature sows (Great York × Landrace) were housed in individual pens and, after equal distribution relative to parity (1–6), were allocated to four dietary treatments (12 sows per treatment).[23] Gestation and lactation diets, based on maize and soya bean meal with a total phosphorus content of 0.42 % (gestation diet) or 0.51 % (lactation diet) were either not supplemented (negative control group) or supplemented with Axtra® PHY phytase to provide 2 000 FTU/kg of diet (1× group) or 200 000 FTU/kg of diet (100× group). Positive control diets with total phosphorus contents of 0.64 % (gestation) or 0.71 % (lactation) were also considered. Sows were under assessment for a full reproduction cycle (from weaning to weaning; a total of 143 days). Sows were fed, restrictively, a gestation diet (pelleted feed) from the start of the study until day 108 and a lactation diet (pelleted feed) from day 109 until the end of the experiment. Sow body weight and back-fat thickness were measured at weaning, at mating (start of experiment), at day 109 of gestation, at farrowing and at weaning (end of experiment). The feed intakes were recorded daily, and changes in body weight and back-fat thickness were calculated for several periods with respect to the above measurements. For each sow, gestation length, the number of piglets born (live and stillborn), piglet weight, mortality and daily weight gain during lactation, were recorded. An ANOVA was performed with the data considering the sow as the experimental unit. Mean values of the groups were compared with the LSD test. Differences were considered significant at a level of at least $P < 0.05$.

No sows died during the experiment. Two sows (from the positive control and 100× groups) were treated with antibiotics because of low feed intakes and another sow (from the 100× group) was removed from the experiment because of an abortion. There were no significant treatment-related effects on sow feed intake, body weight or back-fat thickness, or any related changes throughout the study. The total number of live-born piglets (mean of 15 piglets per sow) was no different between treatments and the percentage of stillborn piglets was lower in the phytase supplemented groups than the negative control group (10.5%, 4.3 % and 2.5 % for the negative control, 1× and 100× groups, respectively). The number of piglets per litter after cross-fostering was 13.6 and at weaning was 11.7 (mortality ≈ 14 %), and the daily weight gain of the piglets was 0.255 kg; no differences were found between treatments. The supplementation of the experimental diets with the phytase contained in Axtra® PHY up to 100-fold the maximum recommended dose did not have a negative effect on the performance of sows for reproduction. Therefore, the FEEDAP Panel concludes that the additive is safe for sows.

[23] Technical dossier/Section III/Annex III.4.

3.2.7. Conclusions on the safety for target species

The tolerance trials provided in chickens and turkeys for fattening, laying hens, weaned piglets and sows showed that the animals can tolerate doses up to 100-fold the maximum recommended dose of 2 000 FTU/kg feed. Therefore, the FEEDAP Panel concludes that the additive is safe for these target species at the maximum recommended dose.

The Panel considers that the conclusions reached for the chickens and turkeys for fattening can be extended to chickens reared for laying and turkeys reared for breeding, and the conclusions for laying hens can be extended to breeding hens at the same maximum dose. Similarly, the conclusions for weaned piglets can be extended to pigs for fattening at the same maximum dose.

Based on the wide margin of safety shown, the conclusions can be extrapolated to minor poultry and porcine species at the same maximum dose.

3.3. Safety for the consumer

The 6-phytase concentrate that is used to formulate the final additive was used in the toxicological studies described below.

3.3.1. Genotoxicity, including mutagenicity, studies

3.3.1.1. Bacterial reverse mutation assay

The potential of the concentrate to induce point mutations was assessed in the *Salmonella* Typhimurium strains TA98, TA100, TA102, TA1535 and TA1537 in accordance with OECD Guideline 471.[24] The test material was tested by the preincubation method, both in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver; S9-mix), up to a maximum concentration of 4 680 µg/mL. No biologically relevant increase in the number of revertant colonies was observed after treatment with the test material at any dose level, either in the presence or absence of metabolic activation. The positive controls performed as expected.

3.3.1.2. *In vitro* mammalian chromosome aberration test

A study was carried out to evaluate the potential of the concentrate to induce structural changes in the chromosomes of human peripheral lymphocytes in the presence and absence of metabolic activation, in accordance with OECD Guideline 473.[25] The cultures were treated with eight concentrations of the test material, diluted in cell culture medium, and the three highest concentrations (1 170, 2 340 and 4 680 µg/mL) were analysed for their ability to induce chromosomal aberrations. Two independent tests were performed, with the following experimental schedules: (1) a three-hour treatment followed by a twenty-hour recovery period before cell harvesting, with and without S9-mix; (2) a 20-hour treatment followed by cell harvesting, without S9-mix, and the same schedule as test 1 with S9-mix. The concentration of S9 homogenate used in the second test was two-fold higher than the concentration used in the first test.

A slight, but statistically significant, increase in the frequency of aberrant metaphases was observed in one culture treated at the intermediate concentration (2 340 µg/mL) without S9-mix in the first test, but it was not reproduced in the duplicate culture and was not dose related. In the second test without S9-mix, small increases in aberrations were observed at 1 170 and 2 340 µg/mL, but they were not dose related and were not confirmed in the duplicate culture. The other frequencies of aberrant metaphases were comparable with the negative controls. No increase was observed in any other experiment in the presence of S9-mix. The positive controls performed as expected.

[24] Technical dossier/Section III/Annex 7.
[25] Technical dossier/Section III/Annex 8.

3.3.2. Sub-chronic repeated dose oral toxicity study

The study was conducted in compliance with OECD Guideline 408 (1998).[26] The test material was diluted with sterile water to give doses of 0, 3.84, 9.6 or 28.8 mg/mL of total protein. Dilutions were prepared weekly and concentrations were confirmed by analysis of frozen samples from weeks 1, 7 and 13. Groups of 10 Sprague–Dawley (Ntac:SD) rats were given test material by gavage at a dose of 0, 19.2, 48 or 144 mg total protein/kg body weight per day. Doses were administered in a constant volume of 5 mL/kg body weight. Animals were inspected twice daily and examined in detail weekly. On one occasion towards the end of the study, all animals were examined for reactivity, grip strength and motor activity. Body weight, food and water intake were recorded weekly throughout the study. Ophthalmoscopy was conducted on all animals prior to the start of the study and on all animals from the control and high dose groups during the last week of treatment. Blood samples were taken on day 30 or 31 and at the end of the treatment period and were subject to haematological[27] and clinical chemistry[28] examinations. Urine samples collected from all groups during the final week of treatment were examined.[29] All animals were subject to necropsy, by which all organs were examined macroscopically and samples were preserved for histological examination. The following organs were weighed as appropriate: adrenals, brain, epididymis, heart, kidneys, liver, ovaries, spleen, testes, thymus and uterus.

There were no treatment-related effects on observations, body weight, food intake, water intake, ophthalmoscopy or urinalysis. Some differences were seen between groups in the results of the haematological and clinical chemistry examinations but these were either restricted to one sex only, seen in the low or middle dose level only, or not seen consistently throughout the study. Therefore, none of these findings was considered to be attributable to treatment. There were no treatment-related statistically significant differences in organ weights between treated and control animals. A slight trend towards an increase in testis weight with dose was observed, but was not considered to be related to treatments as there was no associated histopathology. There were no treatment-related effects on the incidence or severity of any lesions in any treated group.

3.3.3. Conclusions on the safety for the consumer

The results obtained with 6-phytase concentrate used to formulate the additive in the genotoxicity studies and in the sub-chronic oral toxicity study do not indicate any reason for concern for consumer safety arising from the use of the product as a feed additive.

3.4. Safety for the user

The studies provided in this section were performed either with the 6-phytase concentrate that is used to formulate the final additive or with an intermediate product (referred to as "6-phytase intermediate") that consists of the enzyme concentrate, sodium benzoate and potassium sorbate.

3.4.1. Effects on the respiratory system

The acute inhalation toxicity of "6-phytase intermediate" was investigated by exposing a group of five male and five female Sprague–Dawley rats to an aerosol atmosphere using a chamber concentration of 5.0 mg/L of the test material.[30] Based on the results, the test material is classified as non-toxic by inhalation.

[26] Technical dossier/Section III/Annex 9.
[27] Including: haemoglobin, red blood cell counts, haematocrit, MCV, MCH, MCHC, white blood cell count (including differential counts), platelet counts, activated partial thromboplastin time, prothrombin time and fibrinogen.
[28] Including: AST, ALT AP, γGT, bilirubin, cholesterol, triglycerides, urea, creatinine, glucose, sodium, potassium, calcium, magnesium, inorganic phosphorus, chloride, total protein, albumin and globulin (and the ratio of globulin to albumin).
[29] Including: volume, sodium, potassium, chloride, specific gravity, pH, colour, protein, leucocytes, nitrite, blood, glucose, ketones, bilirubin and uribilinogen.
[30] Technical dossier/Section III/Annex 10.

Because of the proteinaceous nature of the active substance, the additive should be considered a potential respiratory sensitiser.

3.4.2. Effects on skin and eyes

The acute dermal irritant potential of the 6-phytase concentrate was investigated in three rabbits, in accordance with OECD Guideline 404.[31] Based on the results, the test material is classified as non-irritant to skin.

The eye irritancy of the 6-phytase concentrate was investigated in accordance with the method recommended in OECD Guideline 405.[32] On the basis of the results, the test material is classified as non-irritant to eyes.

A study was performed to assess the skin sensitisation potential of the "6-phytase intermediate" in the CBA/Ca mouse strain after topical application to the dorsal surface of the ear, in accordance with OECD Guideline 429.[33] The stimulation index for the three concentrations of the test material was always lower than 3; therefore, the test material is considered to be a non-sensitiser under the tested conditions.

3.4.3. Conclusions on the safety for the user

The 6-phytase concentrate or an intermediate product were tested. The results showed that the test materials are not toxic by inhalation, not irritant to skin or eyes and not dermal sensitisers. The Panel considers that the substances added during the formulation of the additive are not likely to contribute to sensitisation, but the presence of sodium chloride may influence the irritant properties. Therefore, the additive is not considered a dermal sensitiser but should be considered a potential irritant to skin, eyes and the respiratory tract.

Because of the proteinaceous nature of the active substance, the additive is considered a potential respiratory sensitiser.

3.5. 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 concerns associated with the genetic modification of the production strain.

The active substance of the additive is a protein, and as such will probably 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.

4. Efficacy

None of the test materials used in the efficacy studies was the final product, but were preparations with a lower enzyme activity (two- to three-fold lower). The Panel considers these test materials to be adequate and will refer to these materials in the studies below as Axtra® PHY phytase.

In all of the trials, differences were considered statistically significant at a level of at least $P < 0.05$.

[31] Technical dossier/Section III/Annex 11.
[32] Technical dossier/Section III/Annex 12.
[33] Technical dossier/Section III/Annex 13.

4.1. Chickens for fattening

4.1.1. Trial 1

A digestibility/balance trial was performed with a total of 153 male chickens (Ross).[34] During their first week of life, the birds were fed a common diet (total phosphorus content of 0.84 %), then the birds were fed one of the three experimental diets. Until day 14, the birds were kept in three pens of 17 birds per treatment. On day 14, a total of 144 birds were randomly selected and transferred, in groups of four birds, to cages, resulting in 12 replicates per treatment. A basal diet, based on wheat and soya bean meal with a total phosphorus content of 0.73 % (calcium 1.1 %), was either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 250 FTU/kg feed (confirmed by analysis). A positive control diet was also considered (total phosphorus content 0.84 %; calcium 1.1 %). Feed was offered *ad libitum* in pelleted form and contained titanium dioxide as an external marker. The performance of the birds was monitored throughout the study. The balance study consisted of three days of adaptation to the cage (days 14 to 17), and four days of excreta collection and feed intake measurements (days 18 to 21). Birds were weighed on days 0, 7, 14 and 21 (individually identified). On day 21, birds were slaughtered and ileal contents were collected and pooled per cage. Ileal contents, excreta and feed samples were analysed in order to study the digestibility/utilisation of dry matter (data not shown), phosphorus and calcium, as well as the energy content (not analysed in ileal contents, data not shown). An ANOVA was performed with the data and the comparison of the group means was performed using a Tukey test.

Two birds died in the pre-experimental phase. Ileal digestibility and retention of phosphorus were significantly higher in the group fed the phytase at 250 FTU/kg feed than in the group fed the negative control diet (Table 2).

Table 2: Effect of Axtra® PHY on the ileal digestibility and retention of dietary phosphorus and calcium

Treatment	Ileal digestibility (%)		Retention (%)	
	Phosphorus	Calcium	Phosphorus	Calcium
Negative control	75.3[b]	76.4	56.8[b]	56.4[b]
250 FTU/kg	83.1[a]	78.1	63.9[a]	64.8[a]
Positive control	74.9	75.7	55.7[b]	64.0[a]

[a,b] Values within one column with different superscripts are significantly different ($P < 0.05$).

4.1.2. Trial 2

A balance trial was performed with a total of 160 male chickens (Ross 308).[35] During their first week of life, the birds were fed a commercial diet. From days 7 to 21, the birds were distributed among raised floor pens in groups of four birds and allocated to one of the five dietary treatments (resulting in eight replicates per treatment). A basal diet, based on maize and soya bean meal with a total phosphorus content of 0.49 % (calcium 0.95 %), was either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 250, 500 or 1 000 FTU/kg feed (confirmed by analysis). A positive control diet was also considered (total phosphorus content 0.65 %; calcium 0.92 %). Feed was offered *ad libitum* in mash form and contained titanium dioxide as an external marker. The performance of the birds was monitored throughout the study. The birds were weighed on days 0, 7 and 21. The balance study consisted of 10 days of adaptation (days 8 to 18), and three days of total excreta collection and feed intake measurements (days 19 to 21). Excreta and feed samples were analysed in order to study the utilisation of dry matter, phosphorus, calcium and nitrogen (data not shown), as well as the energy content (data not shown). ANOVA was performed with the data, considering the pens as the experimental unit, and the comparison of the means was performed using a Tukey test.

[34] Technical dossier/Section IV/Annex IV.1 and Supplementary information April 2015/Annex IV.1.1.
[35] Technical dossier/Section IV/Annex IV.2 and Supplementary information April 2015/Annex IV.2.1.

Two animals from the 1 000 FTU/kg feed group died. A higher retention of phosphorus was found in birds fed the diet supplemented with 250 FTU/kg feed or more than in the birds fed the negative control diet (Table 3).

Table 3: Effect of Axtra® PHY on the retention of dietary phosphorus and calcium

Treatment	Phosphorus (%)	Calcium (%)
Negative control	46.8[b]	47.5[b]
250 FTU/kg	61.5[a]	62.1[ab]
500 FTU/kg	60.2[a]	63.8[a]
1 000 FTU/kg	64.5[a]	62.5[ab]
Positive control	53.7[ab]	63.4[a]

[a,b] Values within one column with different superscripts are significantly different (P < 0.05).

4.1.3. Trial 3

A digestibility/balance trial with measurements of bone mineralisation was performed with a total of 288 male chickens (Ross 308).[36] Until day 5 of their lives, the birds were housed in a floor pen and were fed a commercial diet. On day five, the birds were transferred to 18 cages, in groups of 16 birds per cage, and were allocated to one of the three dietary treatments, resulting in six replicates per treatment. A basal diet, based on wheat and soya bean meal with a total phosphorus content of 0.43 % (calcium 0.70 %), was either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 250 FTU/kg feed (confirmed by analysis). A positive control diet was also considered (total phosphorus content 0.61 %; calcium 0.80 %). Feed was offered *ad libitum* in pelleted form and contained titanium dioxide as an external marker. The birds were weighed on days 5 and 21, and feed intake was measured throughout the study. The balance study consisted of three days of excreta collection and feed intake measurements (days 18 to 20). On day 21, the birds were slaughtered and ileal contents were collected and pooled per cage; the left tibia was collected from four birds per cage. Ileal contents, excreta and feed samples were analysed in order to study the digestibility/utilisation of dry matter, phosphorus and calcium. Bones were dried, defatted and then ash content was determined. An ANOVA was performed with the data, considering the pen as the experimental unit, and the comparison of the group means was performed using a Tukey test.

Phosphorus ileal digestibility, phosphorus retention and tibia ash content were higher in the group fed the phytase at 250 FTU/kg feed than in group fed the negative control diet (Table 4).

Table 4: Effect of Axtra® PHY on the apparent ileal digestibility and the retention of phosphorus and calcium, and on the tibia ash content

Treatment	Ileal digestibility (%)		Retention (%)		Tibia ash content (g/kg DM fat free)
	Phosphorus	Calcium	Phosphorus	Calcium	
Negative control	59.1[b]	68.1[a]	56.7[b]	44.9[b]	380[c]
250 FTU/kg	65.5[a]	64.0[a]	64.0[a]	46.7[b]	419[b]
Positive control	55.8[c]	48.3[b]	53.0[b]	52.2[a]	465[a]

[a,b,c] Values within one column with different superscripts are significantly different (P < 0.05).

In three trials, the supplementation of diets with Axtra®PHY at 250 FTU/kg resulted in a significant improvement in phosphorus retention in chickens for fattening.

[36] Technical dossier/Section IV/Annex IV.3 and Supplementary information April 2015/Annex IV.3.1.

4.2. Turkeys for fattening

4.2.1. Trial 1

A balance trial with measurements of bone mineralisation was performed with 450 one-day-old male turkeys (BUT Big 6).[37] Until day seven of life, the birds were housed in eight floor pens and fed a diet with a total phosphorus content of 0.86 %. At day seven, the turkeys were distributed among 45 cages in groups of 10 and allocated to five dietary treatments, resulting in nine replicates per treatment. A basal diet, based on maize and soya bean meal and with a total phosphorus content of 0.63 % (calcium 0.99 %), was either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 250, 1 000 or 2 000 FTU/kg feed (confirmed by analysis). A positive control was also considered (total phosphorus content 0.78 %; calcium 1.16 %). Feed was offered *ad libitum* in pelleted form and contained hydrochloric acid (HCl)-insoluble ash as an external marker. The birds were weighed on days 7 and 29, and feed intake was measured throughout the study. The balance study consisted of four days of excreta collection and feed intake measurements (days 25 to 29). At the end of the study, the birds were slaughtered and the left tibia was collected for four birds per cage. Excreta and feed samples were analysed in order to study the utilisation of dry matter, nitrogen, energy (data not shown), phosphorus and calcium. Bones were dried, defatted and then ash and phosphorus contents were determined. An ANOVA was performed with the data, considering the cage as the experimental unit, and the comparison of the group means was performed using a Tukey test.

The mortality values were 3.3, 4.4, 1.1, 1.1 and 6.7 % for the groups fed the negative control, 250 FTU/kg, 1 000 FTU/kg, 2 000 FTU/kg and positive control diets, respectively, and were not statistically different among treatments. The results showed that groups fed a diet supplemented with phytase (250 FTU/kg or more) had significantly higher phosphorus retention, tibia ash and phosphorus content than in group fed the negative control diet (Table 5).

Table 5: Effect of Axtra® PHY on the retention of phosphorus and calcium and on the tibia ash and phosphorus content

Treatment	Retention (%)		Tibia content (g/kg DM fat free)	
	Phosphorus	Calcium	Ash	Phosphorus
Negative control	47.8[c]	40.9[c]	333[c]	59[c]
250 FTU/kg	58.4[b]	50.5[b]	389[b]	69[b]
1 000 FTU/kg	68.7[a]	61.6[a]	443[a]	81[a]
2 000 FTU/kg	66.5[a]	59.4[a]	447[a]	80[a]
Positive control	47.6[c]	43.1[c]	401[b]	71[b]

[a,b,c] Values within one column with different superscripts are significantly different ($P < 0.05$).

4.2.2. Trial 2

A digestibility/balance trial with measurements of bone mineralisation was performed with a total of 400 one-day-old male turkeys (Nicholas).[38] Birds were distributed into 40 cages in groups of 10 birds and allocated to one of the five dietary treatments (representing eight replicates per treatment). A basal diet, based on maize and soya bean meal with a total phosphorus content of 0.74 % (calcium 1.36 %) was either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 250, 500 or 1 000 FTU/kg feed (confirmed by analysis). A positive control was also considered (total phosphorus content 0.92 %; calcium 1.66 %). Feed was offered *ad libitum* in pelleted form and contained HCl-insoluble ash as an external marker. The birds were weighed on days 0, 7, 14, 21 and 27 or 28, and feed intake was measured throughout the study. The balance study consisted of three days of excreta collection and feed intake measurements (days 22 to 24). Excreta and feed samples were analysed in order to study the retention of phosphorus and calcium. An ANOVA was performed

[37] Technical dossier/Section IV/Annex IV.7 and Supplementary information April 2015/Annex 7.1.
[38] Technical dossier/Section IV/Annex IV.8 and Supplementary information April 2015/Annex IV.8.1.

with the data, considering the cage as the experimental unit, and the comparison of the group means was performed using a Tukey test.

The addition of phytase to the diets resulted in a significantly higher phosphorus digestibility/retention at 250 FTU/kg feed or above (Table 6).

Table 6: Effect of Axtra® PHY on the retention of phosphorus

Treatment	Retention (%)	
	Phosphorus	Calcium
Negative control	41.8[c]	25.6[b]
250 FTU/kg	50.6[b]	40.1[a]
500 FTU/kg	60.3[a]	37.2[a]
1 000 FTU/kg	58.7[a]	45.6[a]
Positive control	34.4[c]	14.8[c]

[a,b,c] Values within one column with different superscripts are significantly different ($P < 0.05$).

4.2.3. Trial 3

A digestibility trial with measurements of bone mineralisation was performed with 288 one-day-old male turkeys (BUT Big 6).[39] Until day five of life, the birds were housed in eight floor pens and fed a commercial diet. At day five, the turkeys were distributed among 18 cages in groups of 16 and allocated to three dietary treatments, resulting in six replicates per treatment. A basal diet, based on maize and soya bean meal and with a total phosphorus content of 0.56 % (calcium 0.80 %), was either not supplemented (negative control) or supplemented with Axtra® PHY phytase at 250 FTU/kg feed (confirmed by analysis). A positive control was also considered (total phosphorus content 0.75 %, calcium 0.90 %). Feed was offered *ad libitum* in pelleted form and contained titanium dioxide as an external marker. The birds were weighed at the beginning and at the end of the study, and feed intake was measured throughout the study. At the end of the study (day 21), the birds were slaughtered and ileal samples from all animals (pooled per cage) and the left tibia from four birds per cage were collected. Ileal and feed samples were analysed in order to study the utilisation of dry matter (data not shown), phosphorus and calcium. Bones were dried, defatted and then ash content was determined. An ANOVA was performed with the data, considering the cage as the experimental unit, and the comparison of the group means was performed using a Tukey test.

Apparent ileal digestibility of phosphorus and tibia ash content were significantly higher in the group fed the diet supplemented with phytase at 250 FTU/kg than in the negative control group (Table 7).

Table 7: Effect of Axtra® PHY on the ileal digestibility of phosphorus and calcium and on the tibia ash content

Treatment	Ileal digestibility (%)		Tibia ash content (g/kg DM fat free)
	Phosphorus	Calcium	
Negative control	49.8[b]	51.2	363[c]
250 FTU/kg	59.5[a]	56.5	414[b]
Positive control	59.6[a]	53.2	480[a]

[a,b,c] Values within one column with different superscripts are significantly different ($P < 0.05$).

In three trials, the supplementation of diets with the additive at the dose of 250 FTU/kg resulted in a significant improvement of phosphorus utilisation in turkeys for fattening.

[39] Technical dossier/Section IV/Annex IV.9 and Supplementary information April 2015/Annex IV.9.1.

4.3. Laying hens

A total of five trials were assessed. Two[40] of these studies did not meet the necessary requirements because either the retention of phosphorus was not measured or the performance of the hens was not reported; consequently, these two studies were not considered further in the assessment. Two short-term trials and one long-term trial were considered.

4.3.1. Trial 1

A balance trial was performed with a total of 72 21-week-old laying hens (Hy-line) (90 % laying rate).[41] The trial comprised three dietary treatments with 12 replicates of two hens each (two hens/cage). From week 21 to week 27 of life, hens were fed with a basal diet, based on wheat and soya bean meal with a total phosphorus content of 0.34 % (calcium 3.4 %), that was either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 250 FTU/kg feed (confirmed by analysis). A positive control was also considered (total phosphorus content 0.65 %; calcium 3.6 %). Feed was offered *ad libitum* in mash form. The balance study consisted of three days of total excreta collection during the last week of the trial. Feed intake was measured during the excreta collection period. Laying performance (laying rate, egg weight and feed-to-gain ratio) and feed intake were measured throughout the study. Hens were weighed at the start and at the end of the trial. An ANOVA was performed with the data, considering the cage as the experimental unit, and the comparison of the group means was performed using a Tukey test.

Phosphorus retention (measured in the excreta) was significantly higher in the group fed the diet supplemented with 250 FTU/kg feed than in the group fed the negative control diet. The results also showed that the laying rate, egg mass production and feed intake were not different in the group fed the phytase at 250 FTU/kg, as compared with the negative control, between weeks 21 and 27.

Table 8: Effect of Axtra® PHY on the retention of phosphorus and calcium[1]

Treatment	Phosphorus (%)	Calcium (%)
Negative control	21.8 [b]	40.8 [b]
250 FTU/kg	39.9 [a]	51.5 [a]
Positive control	27.1 [b]	44.9[ab]

[1] Retention values do not consider the P in the eggs.
[a,b] Values within one column with different superscripts are significantly different ($P < 0.05$).

4.3.2. Trial 2

A balance study was performed with 36 24-week-old ISA Brown hens that were individually caged and allocated to one of the two dietary treatments, resulting in 18 replicates per treatment.[42] A basal diet, based on wheat, barley, maize and soya bean meal with a total phosphorus content of 0.34 % (calcium 3.9 %), was either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 250 FTU/kg feed (confirmed by analysis). Experimental feeds were offered for 28 days in mash form and contained chromium oxide as an external marker. The body weight of the hens was measured at the start and at the end of the trial. Feed intake was recorded weekly, egg production and egg weight were measured daily, and egg mass and feed efficiency were calculated weekly. The collection of excreta samples was carried out for three days (days 26 to 28) and these were analysed for phosphorus and calcium. All eggs laid on days 25 to 27 were collected, pooled per bird and analysed for phosphorus content. At the end of the study, all of the hens were slaughtered and the left tibia collected for the determination of ash, calcium and phosphorus contents. An ANOVA was performed with the data, considering the cage as the experimental unit, and the comparison of the group means was performed using a Tukey test.

[40] Technical dossier/Section IV/Annexes IV.4 and IV.5.
[41] Technical dossier/Section IV/Annexes IV.6 and Supplementary information April 2015/Annex.
[42] Technical dossier/Supplementary information April 2015/Annex IV.20.

The retention of phosphorus, measured in the excreta, and the tibia ash/phosphorus contents were significantly increased by the phytase (Table 9). The phosphorus content of eggs was not modified by the treatments. During the four weeks of the trial, no differences were found between the treatments with regard to feed intake, egg production (98 %) or egg weight (64 g).

Table 9: Effect of Axtra® PHY retention of phosphorus and calcium, egg phosphorus content and the tibia ash content

Treatment	Retention (%)		Egg phosphorus content (%)	Tibia (% as is)	
	Phosphorus	Calcium		Ash	Phosphorus
Negative control	30.5[b]	63.9[b]	0.131	22.8[b]	3.9[b]
250 FTU/kg	46.2[a]	70.7[a]	0.133	24.2[a]	4.2[a]

[a,b] Values within one column with different superscripts are significantly different (P < 0.05).

4.3.3. Trial 3

A performance trial was carried out with a total of 240 23-week-old ISA Brown hens. From week 23 to week 26 the egg production was monitored. On week 26 and considering the egg production of the previous three weeks, the hens were distributed to 48 cages in groups of five hens and allocated to one of the three experimental treatments, resulting in 16 replicates per treatment.[43] From week 26 to week 60, hens were fed with two basal diets (changed during week 43), based on maize and soya bean meal with a total phosphorus content of 0.42 and 0.40 %, respectively (calcium 3.5 and 3.7 %, respectively), that were either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 300 FTU/kg feed (confirmed by analysis). A positive control diet was also considered (total phosphorus content 0.60 and 0.57 %, and calcium 3.6 and 3.8 %, respectively). Feed was offered *ad libitum* in mash form for 34 weeks. The health of the laying hens was monitored daily. Egg production was recorded daily, all eggs produced in one day were weighed every 14 days and total egg mass was calculated. Feed intake was monitored throughout the study and the body weight of the hens was recorded at the beginning and at the end of the trial. Feed to egg mass ratio was calculated. An ANOVA was performed with the data and the comparison of the group means was performed using a Tukey test.

Nine hens in total died, four from the positive control and five from the negative control groups. Feed intake was not affected by treatment. The results showed a significantly higher egg weight and egg mass production, and a better feed to egg mass ratio, in the hens fed the phytase than in the negative control hens.

Table 10: Effect of Axtra® PHY on the performance of hens from weeks 26 to 60

Treatment	Daily feed intake (g)	Laying rate (%)	Egg weight (g)	Egg mass production (g/hen/day)	Feed to egg mass ratio
Negative control	116	89.0	64.7[b]	56.6[b]	2.01[a]
300 FTU/kg	117	92.4	67.2[a]	62.2[a]	1.88[b]
Positive control	119	91.1	66.7[a]	60.0[a]	1.97[a]

[a,b] Values within one column with different superscripts are significantly different ($P < 0.05$).

The supplementation of the diets with 250 FTU/kg feed improved phosphorus utilisation in only two trials. In the third trial, the supplementation of the diets with 300 FTU/kg improved the laying performance of the hens and the feed to egg mass ratio.

[43] Technical dossier/Supplementary information April 2015/Annex IV.19.

4.4. Weaned piglets

4.4.1. Trial 1

A balance trial was performed with a total of 20 castrated male and female weaned piglets (Talent × (Great York × Landrace)), approximately 27 days old and initial body weight of 8.5 kg).[44] During the first week post weaning, the piglets were penned in pairs and fed a commercial diet. During the experimental periods, from days 9 to 22 (period 1: 9 castrated male and 11 female weaned piglets) and from days 23 to 37 (period 2: 12 castrated male and 8 female weaned piglets) post weaning, the piglets were individually housed in pens that permitted a separate collection of faeces and urine. Feed was restricted to provide 3.2 × energy for maintenance, and water was available *ad libitum* over an experimental period of 28 days. Each piglet received different diets over the two periods. A total of five dietary treatments were considered, resulting in eight replicates per treatment (four per period). A basal diet, based on barley, maize and soya bean with a total phosphorus content of 0.47 % (calcium 0.64 %), was either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 250, 500 or 1 000 FTU/kg of diet (confirmed by analysis). A positive control diet was also used (total phosphorus content 0.63 %, calcium 0.71 %). Each period consisted of 10 days of adaptation to the diet and four days of collection of faeces and urine (urine collected in acid). Body weight and feed intake were recorded at days 8, 22 and 37 post weaning, and daily feed intake, daily body weight gain and feed to gain ratio were calculated for period 1 (9–22 days post weaning) and period 2 (23–37 days post weaning). Mortality was checked daily. Faecal and urine samples were collected from days 10 to 14 in both periods (i.e. days 18–22, in period 1, and days 33–37, in period 2, post weaning). Faecal apparent digestibility of dry matter, organic matter, crude protein, crude fat, ash, gross energy, calcium, phosphorus and sodium were determined using HCl-indigestible ash as an external marker. Urine was analysed for energy, nitrogen, calcium and phosphorus. The retention of energy, nitrogen (data not shown), calcium and phosphorus was calculated. An ANOVA was carried out with the data and the group means were compared with a Tukey test.

No pig died during the experiment. Phytase supplementation from 250 FTU/kg significantly increased faecal digestibility of ash, calcium and phosphorus (data not shown), as well as calcium and phosphorus retention (Table 11). Apparent faecal digestibility of dry matter was significantly increased at 500 FTU/kg. Apparent faecal digestibility and retention of the other nutrients tested, as well as the metabolisable energy content of the diets, were not affected by dietary treatments.

Table 11: Effect of Axtra® PHY on the retention of calcium and phosphorus

Treatment	Phosphorus (%)	Calcium (%)
Negative Control	40.7^{d}	49.4^{c}
250 FTU/kg	56.2^{b}	65.9^{ab}
500 FTU/kg	60.0^{ab}	68.5^{ab}
1 000 FTU/kg	64.5^{a}	72.7^{a}
Positive control	50.0^{c}	59.7^{b}

^{a,b,c,d} Values within one column with different superscripts are significantly different ($P < 0.05$).

4.4.2. Trial 2

A balance trial was performed with a total of 54 castrated male weaned piglets (PIC commercial breed, approximately 33 days old and initial body weight of 9.8 kg) housed in individual pens, before and after moving to digestibility crates, and allocated to six dietary treatments (nine pigs per treatment).[45] A basal diet, based on maize and soya bean meal with a total phosphorus content of 0.45 % (calcium 0.68 %), was either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 250, 500, 1 000 or 2 000 FTU/kg of diet (confirmed by analysis). A positive

[44] Technical dossier/Section IV/Annex IV.16 and Supplementary information April 2015/Annex IV.16.1 and IV.16.2.
[45] Technical dossier/Section IV/Annex IV.17 and Supplementary information April 2015/Annex IV.17.1 and Annex IV.17.2.

control group was also considered (total phosphorus content 0.68 %, calcium 0.89 %). The overall experimental period lasted 42 days and was conducted in three runs (14 days for each run). Each run (period) consisted of 10 days of adaptation to the diet and four days of collection of faeces and urine (which were separated). Test diets (pelleted feed) were restricted to provide 3 × metabolisable energy for maintenance, and water was available *ad libitum*. Feed intake and body weight were recorded at days 1 and 14 of each run, and daily feed intake, daily weight gain and feed-to-gain ratio were calculated for each run. Mortality was checked daily. Faecal apparent digestibility of dry matter, crude protein, gross energy, calcium and phosphorus were determined using HCl-acid indigestible ash as an external marker. Urine was analysed for gross energy, nitrogen, calcium and phosphorus. Retention of gross energy, nitrogen, calcium and phosphorus was calculated. An ANOVA was carried out with the data and the group means were compared with a Tukey test.

No pig died during the experiment. Phytase supplementation from 250 FTU/kg significantly increased apparent faecal digestibility (data not shown) and retention of phosphorus compared with the negative control diet (Table 12). Significant improvements with regard to calcium digestibility, at the dose of 1000 FTU/kg (data not shown), and retention, at the dose of 500 FTU/kg feed, were found. Apparent faecal digestibility of dry matter, crude protein and gross energy, and retention of nitrogen and gross energy, were not affected by dietary treatments (data not shown).

Table 12: Effect of Axtra® PHY on the retention of phosphorus and calcium

Treatment	Phosphorus (%)	Calcium (%)
Negative control	51.5^{d}	39.8^{d}
250 FTU/kg	59.4^{c}	41.2^{cd}
500 FTU/kg	62.8^{bc}	49.6^{bc}
1 000 FTU/kg	76.3^{a}	60.2^{a}
2 000 FTU/kg	75.5^{a}	60.2^{a}
Positive control	68.3^{b}	58.2^{ab}

^{a,b,c,d} Values within one column with different superscripts are significantly different ($P < 0.05$).

4.4.3. Trial 3

A balance trial was performed with a total of 22 castrated male and 18 female weaned piglets (Great York × Landrace mated with Tempo commercial hybrid boar, approximately 35–42 days old with an initial body weight of 8.1 kg).[46] The study was performed in two runs; 20 piglets participated in run 1 and 20 piglets were used in run 2. During the first 12 days post weaning, the piglets were penned in litter groups and fed a commercial diet. On day 13 post weaning, piglets were moved to individual digestibility crates and allocated to four dietary treatments (in total, 10 piglets were used per treatment). Feed was restricted to provide 3.5 × energy for maintenance (at day 19 post weaning) and water was available *ad libitum* over an experimental period of 12 days. Pigs were fed a weaner diet (pelleted feed) from day 13 until day 24 post weaning. The diets were produced as pellets but, in order to ensure a sufficient feed intake, the pellets were mixed with water, at a water-to-pellet ratio of 3:1, just before feeding. The weaner diet, based on barley, maize and soya bean meal with a total phosphorus content of 0.45 % (calcium 0.60 %), was either not supplemented or supplemented with Axtra® PHY phytase to provide 250 or 500 FTU/kg of diet (confirmed by analysis). A positive control diet was also considered (total phosphorus content 0.63 %, calcium 0.70 %). The experiment consisted of seven days of adaptation to the diet and five days (day 19 until day 24 post weaning) of collection of faeces and urine (urine collected in acid). Feed intake and body weight were recorded at days 12 and 24 post weaning, and daily feed intake, daily weight gain and feed to gain ratio were calculated. Mortality was checked daily. Faecal apparent digestibility of dry matter, organic matter, crude protein, crude fat, ash, gross energy, calcium, phosphorus and sodium were determined using HCl-insoluble ash as an external marker. Urine was analysed for nitrogen, calcium and phosphorus, and retention of

[46] Technical dossier/Section IV/Annex IV.18 and supplementary information April 2015/Annex IV.18.1 and Annex IV.18.2.

nitrogen, calcium and phosphorus was calculated. An ANOVA was carried out with the data and the group means were compared with a Tukey test

No pig died during the experiment. Phytase supplementation significantly increased faecal digestibility of ash, calcium and phosphorus (data not shown), as well as calcium and phosphorus retention at the dose of 250 FTU/kg or above (Table 13). Faecal digestibility of dry matter, crude protein and gross energy, and retention of nitrogen, was not affected by dietary treatments (data not shown).

Table 13: Effect of Axtra® PHY on the retention of phosphorus and calcium

Treatment	Phosphorus (%)	Calcium (%)
Negative Control	29.8[c]	43.1[b]
250 FTU/kg	59.1[ab]	61.1[a]
500 FTU/kg	61.6[a]	61.6[a]
Positive Control	57.0[b]	47.5[b]

[a,b,c,d] Values within one column with different superscripts are significantly different ($P < 0.05$).

The results of the three short-term trials carried out with weaned piglets showed that the supplementation of the diets with 250 FTU/kg feed increased the phosphorus retention in weaned piglets.

4.5. Pigs for fattening

A total of six trials were assessed, one[47] of which was not considered further in the assessment because only digestibility was measured. Therefore, for pigs for fattening, four short-term were considered and one long-term efficacy studies.

4.5.1. Trial 1

A balance trial was performed with a total of 30 male pigs (Landrace × Piétrain, approximately 105 days old and initial body weight of 48 kg) housed individually and allocated to five dietary treatments.[48] A basal diet, based on barley, wheat and soya bean with a total phosphorus content of 0.38 % (calcium 0.60 %), was either not supplemented (negative control) or supplemented with Axtra®PHY phytase to provide 250, 1 000 and 2 000 FTU/kg feed (confirmed by analysis). A positive control was also considered (total phosphorus content 0.53 %; calcium 0.68 %). The balance study was conducted seven times, using 10 individually caged animals in each run (20 pigs used two times and 10 used three times). Each run consisted of 10 days of adaptation to the diet and three days of collection. Between runs, the animals received a commercial diet for one week. Animals changed diets when involved in a second/third round. Pigs were housed individually in crates for a three-day adjustment period prior to a three-day collection period of faeces and urine (urine collected in acid). The overall experimental period lasted 63 days. Test diets (mashed feed) were fed restrictively to provide 2.2 × metabolisable energy for maintenance, and water was available *ad libitum*. Mortality was checked daily. Faecal apparent digestibility of dry matter, nitrogen, gross energy, calcium and phosphorus were determined, using three methods (total faeces collection method and methods using titanium dioxide or HCl-indigestible ash as external markers). Urine was analysed for gross energy, nitrogen, calcium and phosphorus. Retention of nitrogen, gross energy, calcium and phosphorus were also determined. An ANOVA was carried out with the data and the group means were compared with a Tukey test.

No pig died during the experiment, but five pigs were removed from the study mainly because of respiratory problems. In total, the number of observations per treatment were 13, 13, 13, 12 and 14 for the negative control, 250, 1 000 and 2 000 FTU/kg feed and positive control groups, respectively. Phytase addition from 250 FTU/kg feed significantly increased faecal digestibility of calcium,

[47] Technical dossier/Section IV/Annex IV.10.
[48] Technical dossier/Section IV/Annex IV.11 and Supplementary information April 2015/Annex IV.11.1.

phosphorus and dry matter (data not shown), as well as calcium and phosphorus retention (Table 14). Faecal digestibility and retention of nitrogen and gross energy were not affected by dietary treatments (data not shown).

Table 14: Effect of Axtra® PHY on the retention of phosphorus and calcium

Treatment	Phosphorus (%)	Calcium (%)
Negative control	35.0[d]	43.8[c]
250 FTU/kg	54.0[bc]	59.2[ab]
1 000 FTU/kg	62.3[ab]	65.3[a]
2 000 FTU/kg	64.6[a]	67.5[a]
Positive control	45.1[c]	54.7[b]

[a,b,c,d] Values within one column with different superscripts are significantly different ($P < 0.05$).

4.5.2. Trial 2

A balance trial was performed with a total of 45 castrated male pigs (Camborough 22 or Line 22 × Terminal Sire Boars, approximately 79 days old and initial body weight of 43 kg) housed individually and allocated to five dietary treatments (nine pigs per treatment).[49] A basal diet, based on maize and soya bean meal with a total phosphorus content of 0.59 % (calcium 0.57 %), was either not supplemented or supplemented with Axtra® PHY to provide 250, 500 or 2 000 FTU/kg feed (confirmed by analysis) A positive control group was also considered (total phosphorus content of 0.71 %; calcium 0.72 %). The overall experimental period lasted 42 days and was conducted in three runs (14 days for each run). Each run consisted of 10 days of adaptation to the diet and four days of collection of faeces and urine (separated). Test diets (mashed feed) were fed restrictively to provide 3 × metabolisable energy for maintenance, and water was available *ad libitum*. Feed intake and body weight were recorded at days 1 and 14 of each run, and average feed intake, daily weight gain and feed to gain ratio were calculated for each run. Mortality was checked daily. Faecal apparent digestibility of dry matter, crude protein, gross energy, calcium and phosphorus were determined using HCl-insoluble ash as an external marker. Retention of nitrogen, gross energy, calcium and phosphorus were also calculated. An ANOVA was carried out with the data and the group means were compared with an LSD test.

No pig died during the experiment, but one pig, from the negative control group, was removed from the study because of severe lameness. Phytase supplementation from 250 FTU/kg feed increased significantly faecal digestibility of calcium and phosphorus (data not shown), and calcium retention, but phosphorus retention increased only from the dose of 500 FTU/kg (Table 15). Faecal digestibility of dry matter, crude protein, gross energy, and retention of gross energy, was not affected by dietary treatments (data not shown).

Table 15: Effect of Axtra® PHY on the retention of phosphorus and calcium

Treatment	Phosphorus (%)	Calcium (%)
Negative control	36.9[cd]	51.4[c]
250 FTU/kg	41.7[bc]	62.4[b]
500 FTU/kg	48.6[a]	68.5[a]
2 000 FTU/kg	46.8[ab]	67.8[a]
Positive control	34.0[d]	52.8[c]

[a,b,c,d] Values within one column with different superscripts are significantly different ($P < 0.05$).

[49] Technical dossier/Section IV/Annex IV.12 and Supplementary information April 2015.

4.5.3. Trial 3

A balance trial was performed with a total of 48 male pigs (Landrace × Piétrain, approximately 98–112 days old and initial body weight of 61.6 kg) housed in growing pens (three pigs/pen), before and after moving to individual digestibility crates, and allocated to four dietary treatments (12 pigs per treatment).[50] A basal diet, based on barley, wheat, maize and soya bean meal with a total phosphorus content of 0.46 % (calcium 0.46 %), was either not supplemented (negative control) or supplemented with Axtra® PHY to provide 250 or 500 FTU/kg of diet (confirmed by analysis). A positive control diet was also considered (total phosphorus content of 0.65 %, calcium 0.55 %). The experiment was performed in four runs. Each run (period) consisted of 10 days of adaptation to the diet and three days of collection of faeces and urine (which were separated; urine collected in acid). Test diets (pelleted feed) were fed on *ad libitum* basis during adaptation in growing pens and restricted to provide 2.2 × metabolisable energy for maintenance when present in the metabolic pens (and mixed with water at a water-to-pellet ratio of 1:2); water was available *ad libitum*. Pigs were weighed and feed intake was monitored throughout the study. Faecal apparent digestibility of dry matter, nitrogen, gross energy, ash, calcium and phosphorus was determined, using three methods (total faeces collection method and methods using titanium dioxide and HCl-indigestible ash as external markers). Retention of gross energy, nitrogen, calcium and phosphorus were also determined. An ANOVA was carried out with the data and the group means were compared with a Tukey test.

No pig died during the experiment. Phytase addition from the dose of 250 FTU/kg feed significantly increased faecal digestibility of phosphorus, ash and calcium (data not shown), as well as phosphorus retention (Table 16). Retention of nitrogen and gross energy were not affected by dietary treatments (data not shown).

Table 16: Effect of Axtra® PHY on the retention of phosphorus and calcium

Treatment	Phosphorus (%)	Calcium (%)
Negative control	33.6^{c}	33.8^{b}
250 FTU/kg	50.2^{ab}	46.7^{ab}
500 FTU/kg	57.9^{a}	56.2^{a}
Positive control	42.3^{bc}	50.1^{a}

^{a,b,c} Values within one column for the same study with different superscripts are significantly different ($P < 0.05$).

4.5.4. Trial 4

A balance trial was performed with a total of eight castrated male pigs (Topigs × Piétrain, initial body weight of 41 kg).[51] The overall experimental period lasted 35 days (seven-day adaptation period and 28-day digestibility study) and followed a 4 × 4 Latin-square design (four periods; eight pigs per treatment). Each period consisted of three days of adaptation to the diet and four days of collection of faeces and urine (separated, total faeces and urine collection). Test diets (mashed feed) were fed restrictively to provide 2.8 × metabolisable energy for maintenance, and water was available *ad libitum*. A basal diet, based on barley, wheat, maize and soya bean meal with a total phosphorus content of 0.36 % (calcium 0.60 %), was either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 250 or 500 FTU/kg feed (confirmed by analysis). A positive control diet was also considered (total phosphorus content of 0.48 %, calcium 0.76 %). Pigs were weighed at the start of the study and every week thereafter. Faecal digestibility of dry matter, calcium and phosphorus were determined, using two methods (total faeces collection method and a method using titanium dioxide as a marker). The retention of phosphorus was also determined. An ANOVA was carried out with the data and the group means were compared with a Tukey test.

No pig died during the experiment. Phytase addition from 250 FTU/kg feed significantly increased faecal digestibility of calcium, phosphorus and dry matter (data not shown), as well as phosphorus

[50] Technical dossier/Supplementary information April 2015/Annex IV.22.
[51] Technical dossier/Supplementary information April 2015/Annex IV.23.

retention (40.6, 48.0, 59.9 and 46.5 % for the negative control, 250 FTU/kg, 500 FTU/kg and positive control groups, respectively).

4.5.5. Trial 5

A performance study with measurements of bone mineralisation was conducted with a total of 36 male and 36 female Piétrain × (Landrace × Duroc) pigs (initial body weight of 30.7 kg) individually housed in 72 pens and allocated to four dietary treatments (18 replicates per treatment).[52] Two grower diets and one finisher diet, based on barley, wheat, maize and soya bean meal with a total phosphorus content of 0.43, 0.49 and 0.46 %, respectively (calcium 0.60, 0.55 and 0.49 %), were either not supplemented (negative control) or supplemented with Axtra®PHY to provide 250 or 500 FTU/kg feed (confirmed by analysis). A positive control was also considered (phosphorus contents of 0.55, 0.54 and 0.50 %, respectively, and calcium contents of 0.70, 0.65 and 0.60 %, respectively). Feed and water were available *ad libitum* over an experimental period of 96 days. Pigs were fed a grower diet (pelleted feed) from day 1 until day 35 (phase 1), a grower diet (pelleted feed) from day 36 until day 70 (phase 2), and a finisher diet (pelleted feed) from day 71 until day 96 (phase 3) of the experiment. Feed intake and body weight were measured at days 0, 35, 70 and 96, and daily feed intake, daily weight gain and feed to gain ratio were calculated for phase 1 (days 1–35 of the experiment), phase 2 (days 36–70 of the experiment), phase 3 (days 71–96 of the experiment) and the overall period. Health status and mortality were checked daily. In addition, on day 96 of the experiment, all pigs were slaughtered for the evaluation of carcass characteristics and bone mineralisation (both metacarpi were extracted). Bones were defatted, dried, weighed and ashed and then analysed for dry matter, ash, calcium and phosphorus contents. Data were statistically analysed by ANOVA and the comparison of the means was performed using a Tukey test.

No pig died during the experiment. Phytase diet supplementation at 250 FTU/kg resulted in a significant improvement in feed to gain ratio (Table 17), but the effect was not found at 500 FTU/kg feed.

Table 17: Effect of Axtra®PHY on the performance of pigs for fattening

Treatment	Daily feed intake (kg)	Final body weight (kg)	Average daily gain (kg)	Feed to gain ratio
Negative control	2.05	100.2[b]	0.72[b]	2.84[a]
250 FTU/kg	2.09	107.2[ab]	0.80[ab]	2.63[b]
500 FTU/kg	2.12	106.6[ab]	0.79[ab]	2.70[ab]
Positive control	2.16	109.9[a]	0.82[a]	2.64[b]

[a,b] Values within one column for the same study with different superscripts are significantly different ($P < 0.05$).

In three short-term trials with pigs for fattening, the supplementation of the diets with Axtra® PHY phytase at a dose of 250 FTU/kg feed increased phosphorus retention.

4.6. Sows for reproduction

4.6.1. Trial 1

A total of 40 gestating sows (Crossbred) were housed in individual pens and, after equal distribution relative to parity (2–8), were allocated to five dietary treatments (eight sows per treatment).[53] Water was available *ad libitum* over the experimental period (at the start of the experiment all sows were between day 70 and day 100 of gestation; in total, 17 days of the experiment were during gestation). Sows were fed a restricted gestation diet (pelleted feed). The gestation diet, based on maize, sunflower and rapeseed with a total phosphorus content of 0.44 % (calcium 0.52 %), was either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 250, 1 000 or

[52] Technical dossier/Supplementary information April 2015/Annex IV.21.
[53] Technical dossier/Section IV/Annex IV.14 and Supplementary information April 2015/Annex IV.14.1.

2 000 FTU/kg feed (confirmed by analysis). A positive control group was also considered (with a total phosphorus content of 0.62 %; calcium 0.68 %). The experiment consisted of 14 days of adaptation to the diet and three days (day 15 until day 17) of collection of faeces. The sows' weights were measured at only the start of the experiment and feed intake was 3.2 kg/day throughout the experiment. Faecal digestibility of dry matter, organic matter, crude protein, ash, gross energy, calcium (data not shown) and phosphorus were determined, using HCl-indigestible ash as an external marker. An ANOVA was performed with the data and the group means were compared with a Tukey test.

None of the sows died during the experiment. Phytase addition from the dose of 250 FTU/kg significantly increased faecal digestibility of phosphorus (24.4, 36.5, 41.0, 39.5 and 32.7 % for the negative control, 250 FTU/kg, 1 000 FTU/kg, 2 000 FTU/kg and positive control groups, respectively).

4.6.2. Trial 2

A total of 56 lactating sows (Crossbred) were housed in individual pens and, after equal distribution relative to parity (2–6), were allocated to seven dietary treatments (eight sows per treatment).[54] Water was available *ad libitum* over the experimental period (from day 4 until day 21 post farrowing; in total, 17 days of the experiment were during lactation). Sows were fed a restricted lactation diet (pelleted feed) from day 4 until the end of the experiment. A lactation diet, based on maize and soya bean meal with a total phosphorus content of 0.45 % (calcium 0.55 %), was either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 250, 500, 750, 1 000 or 2 000 FTU/kg feed (confirmed by analysis). A positive control was also considered (total phosphorus content of 0.63 %). The experiment consisted of 14 days of adaptation to the diet and three days of collection of faeces. Sow body weight was measured at day 108 of gestation and at weaning (six days after the end of faecal collection). Feed intakes were recorded daily. For each sow, the number of piglets born (live and stillborn) and the number of piglets at the start of the experiment and at weaning, was recorded. Faecal digestibility of dry matter, organic matter, crude protein, ash, gross energy, calcium and phosphorus were determined, using HCl-indigestible ash as an external marker. An ANOVA was performed with the data and group means were compared with a Tukey test.

None of the sows died during the experiment. There were no significant treatment-related effects on sows' body weight or feed intake, or parameters related to the piglet litters. Phytase addition from 250 FTU/kg feed increased apparent faecal digestibility of phosphorus (30.1, 44.6, 48.9, 49.1, 50.4, 52.7 and 39.0 % for the negative control, 250 FTU/kg, 500 FTU/kg, 750 FTU/kg, 1 000 FTU/kg, 2 000 FTU/kg and positive control groups, respectively).

4.6.3. Trial 3

A total of 41 lactating sows (Yorkshire or Yorkshire × Landrace crossbred sows), from two farrowing groups (17 in March and 24 in May), were housed in individual pens and, after equal distribution relative to parity (1–6, average of 2.24), were allocated to three dietary treatments (13/15 sows).[55] A lactation diet, based on maize and soya bean meal with a total phosphorus content of 0.46 % (calcium 0.55 %), was either not supplemented (negative control) or supplemented with Axtra® PHY phytase to provide 250 FTU/kg of diet (confirmed by analysis). A positive control diet was also considered (total phosphorus content 0.63 %, calcium 0.75 %). Feed and water were available *ad libitum* over the experimental period (from day 4 until day 19 post farrowing). The experiment consisted of 13 days of adaptation to the diet and three days of collection of faeces. Sow body weight was measured at farrowing, at day 4 and day 19 post farrowing, and at weaning. Feed intake was recorded daily. For each sow, the number of piglets born (live and stillborn), the number of piglets at days 4 and 19, and the number at weaning were recorded. In addition, piglet weight (at birth, at days 4 and 19 of age, and at weaning), mortality and average daily weight gain during lactation were recorded. Faecal digestibility of dry matter, organic matter and nitrogen, crude fat, neutral detergent fibre, ash, gross

[54] Technical dossier/Section IV/Annex IV.13 and Supplementary information April 2015/Annex IV.13.1.
[55] Technical dossier/Section IV/Annex IV.15 and Supplementary information April 2015/Annex IV.15.1.

energy, calcium and phosphorus were determined using titanium dioxide as an external marker. An ANOVA was performed with the data and group means were compared with a Tukey test.

None of the sows died during the experiment. There were no significant treatment-related effects on sow body weight or feed intake, or on parameters related to piglet litters. Phytase addition at 250 FTU/kg feed significantly increased faecal digestibility of phosphorus (37.3, 47.2 and 42.7 % for the negative control, 250 FTU/kg and positive control groups, respectively).

The supplementation of the diets with 250 FTU/kg feed resulted in a higher faecal apparent phosphorus digestibility in sows (either lactating or gestating).

4.7. Conclusions on the efficacy

Based on the results obtained in the efficacy trials provided, the Panel concludes that the additive is efficacious at the dose of 250 FTU/kg feed in chickens and turkeys for fattening, weaned piglets, pigs for fattening and sows. The FEEDAP Panel notes that, for laying hens, two studies showed a significant effect at 250 FTU/kg and one showed an effect at 300 FTU/kg (the lowest dose tested in the latter study). Considering that efficacy has been demonstrated at 250 FTU/kg for all the other species/categories examined and that the difference between 250 and 300 FTU/kg is small, the Panel concludes that for laying hens the effective dose can be established at 250 FTU/kg.

The Panel considers that the conclusions on efficacy can be extended to chickens reared for laying, to turkeys reared for breeding and to turkeys for breeding purposes. The mode of action of phytases is well known and is considered to be similar within avian species: therefore, these conclusions can also be extrapolated to minor poultry species and minor porcine species at the same dose.

5. 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[56] and Good Manufacturing Practice.

CONCLUSIONS

Axtra® PHY 15 000 L does not give rise to safety concerns with regard to the genetic modification of the production strain. Neither the production strain nor its recombinant DNA was detected in the final product obtained from the production strain.

The additive is safe for chickens and turkeys for fattening, laying hens, weaned piglets and sows at the maximum recommended dose of 2 000 FTU/kg feed. The Panel considers that the conclusions can be extended to chickens reared for laying and turkeys reared for breeding, to turkeys for breeding purposes and to pigs for fattening, and can be extrapolated to minor poultry and porcine species at the same maximum dose.

The use of Axtra® PHY 15 000 L as a feed additive gives rise to no concerns for consumers.

The additive is not considered a dermal sensitiser but should be considered as a potential irritant to skin, eyes and the respiratory tract. Owing to the proteinaceous nature of its active substance, the additive is considered a potential respiratory sensitiser.

The use of Axtra®PHY 15 000 L as a feed additive poses no risks to the environment.

Axtra®PHY 15 000 L is efficacious in chickens and turkeys for fattening, laying hens, piglets, pigs for fattening and sows at the dose of 250 FTU/kg feed. These conclusions can be extended to chickens

[56] 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.

reared for laying, to turkeys reared for breeding and to turkeys for breeding purposes, and extrapolated to minor poultry species and minor porcine species at the dose of 250 FTU/kg.

DOCUMENTATION PROVIDED TO EFSA

1. Axtra®PHY 15 000 L for poultry and porcine species. October 2013. Submitted by Danisco UK Ltd.

2. Axtra®PHY 15 000 L for poultry and porcine species. Supplementary information. April 2015. Submitted by Danisco UK Ltd.

3. Axtra®PHY 15 000 L for poultry and porcine species. Supplementary information. July 2015. Submitted by Danisco UK Ltd.

4. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Axtra®PHY 15 000 L.

5. Comments from Member States received through the ScienceNet.

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Nevalainen H, Suominen P and Taimisto K, 1994. On the safety of *Trichoderma reesei*. Journal of Biotechnology, 37, 193–200.

Sheir-Neiss G and Montenecourt BS, 1984. Characterization of the secreted cellulases of *Trichoderma reesei* wild type and mutants during controlled fermentations. Applied Microbiology and Biotechnology, 20, 46–53.

ANNEX

Annex A. Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Axtra®PHY 15 000L[57]

In the current application authorisation is sought under article 4(1) for Axtra® Phy 15000L, under the category/functional 4(a) "zootechnical additives"/"digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the feed additive for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, turkeys for breeding purposes, laying hens, minor avian species, weaned piglets, pigs for fattening, sows for reproduction, and minor porcine species. The active agent of Axtra® Phy 15000L is 6-phytase (EC 3.1.3.26), produced by fermentation of Trichoderma reesei. According to the Applicant, Axtra® Phy 15000L is a liquid preparation with a guaranteed minimum enzyme activity of 15000 U/g. It is intended to be used in premixtures and/or complete feedingstuffs to obtain 6-phytase activities ranging from 250 to 2000 U/kg feedingstuffs. The Applicant uses the international phytase unit defined in the EN ISO 30024, where "one phytase unit (U) is the amount of enzyme which releases one micromole of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37°C".

For the determination of phytase in the feed additive, premixtures and feedingstuffs, the Applicant submitted colorimetric methods derived from the EN ISO 30024 standard method. Phytase is incubated with sodium phytate, resulting in the release of inorganic phosphate and forming a yellow complex with an acidic molybdate/vanadate reagent. Based on the experimental data available, the EURL recommends for official control the in-house validated and further verified method for the quantification of phytase activity in the feed additive and the EN ISO 30024 standard method for the quantification of phytase activity in 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.

[57] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2013-0049-axtraphy15000l.pdf