European Food Safety Authority

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SCIENTIFIC OPINION

Scientific opinion on the efficacy and safety of Quantum® Blue (6-phytase) as a feed additive for poultry (except laying hens) and pigs[1]

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

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 16 February 2018, replaces the earlier version published on 02 October 2013[4]

ABSTRACT

Quantum® Blue is an enzyme preparation (solid and liquid forms) of 6-phytase, produced by a genetically modified strain of *Trichoderma reesei*. The additive is to be used as a feed additive for chickens for fattening/reared for laying, turkeys for fattening/reared for breeding, minor poultry species for fattening and breeding, piglets (weaned), pigs for fattening and sows. Neither the production organism nor recombinant DNA was detected in the final product. The final product does not raise a safety concern with regard to the genetic modification. The results from tolerance studies in chickens and turkeys for fattening, piglets and sows permit to conclude that the additive is safe for those target species at the corresponding maximum recommended dose. The conclusions can be extended to chickens reared for laying and turkeys reared for breeding and to pigs for fattening and can be extrapolated to minor poultry species for fattening or reared for laying/breeding. Based on the results of toxicological studies performed with the fermentation product, the Panel concludes that the additive is of no concern regarding consumer safety. The additive is not irritant to the skin or eyes and is not a skin sensitiser; however, taking account of its proteinaceous nature, the additive is to be considered a potential respiratory sensitiser. No risks to the environment are expected and no further environmental risk assessment is required. Based on the results of the efficacy trials provided, the Panel concludes that the additive has the potential to be efficacious at the dose of 250 phytase units (FTU)/kg feed in poultry species (except layers/breeders), pigs for fattening and sows and at the dose of 500 FTU/kg feed in weaned piglets.

KEY WORDS

zootechnical additive, substances which favourably affect the environment, 6-phytase, *Trichoderma reesei*, genetically modified, poultry, pigs

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[1] On request from European Commission, Question No EFSA-Q-2012-00693, adopted on 11 September 2013.

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[4] This scientific opinion is published following the confidentiality claims submitted by the applicant, in accordance with Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The original version has been removed from the EFSA Journal.

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SUMMARY

Following a request from 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 Quantum® Blue, 6-phytase, as a feed additive for chickens for fattening/reared for laying, turkeys for fattening/reared for breeding, minor poultry species for fattening and breeding, piglets (weaned), pigs for fattening and sows.

The 6-phytase present in the additive is produced by a genetically modified strain of *Trichoderma reesei* and the additive is available in two solid and two liquid forms. The solid and the liquid formulations are considered equivalent in terms of safety and efficacy for the target species, provided that the same dose in enzyme units applies.

Neither the production organism nor recombinant DNA was detected in the final product. The final product does not raise a safety concern with regard to the genetic modification.

Tolerance trials in chickens and turkeys for fattening, weaned piglets and sows were provided. The results showed that the animals tolerated well 100-fold the maximum recommended dosage (2 500 FTU/kg for poultry and 1 750 FTU/kg for pigs). Therefore, the FEEDAP Panel concludes that the additive is safe for those target species at the corresponding maximum recommended dose. The conclusions reached in chickens and turkeys for fattening can be extended to chickens reared for laying and turkeys reared for breeding, provided that the same dose applies; similarly, the conclusions reached in piglets can be extended to pigs for fattening, provided that the same dose applies. Considering the margin of safety demonstrated in major poultry species, the conclusions on the safety for those species can be extrapolated to minor poultry species for fattening or reared for laying/breeding.

The fermentation product that is used to prepare the final formulations of Quantum® Blue tested negative in genotoxicity tests. The results obtained in a subchronic oral toxicity study raised no concerns regarding the product. Therefore, based on the toxicological studies performed with the fermentation product, the FEEDAP Panel concludes that the additive is of no concern regarding consumer safety.

Three final formulations, Quantum® Blue 5 G, 40 P and 10 L, were tested for skin and eye irritation and skin sensitisation. The results showed no evidence of dermal or eye irritation or skin sensitisation. The other final liquid formulation, Quantum® Blue 5 L, was not tested, but based on its composition its irritant potential or skin sensitisation potential is unlikely to be significantly different from that of its tested counterpart (Quantum® Blue 10 L). Taking account of the proteinaceous nature of its active substance, the additive is to be considered a potential respiratory sensitiser.

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

Based on the results of the efficacy trials carried out in chickens and turkeys for fattening, in which the additive significantly improved the phosphorus utilisation, digestibility and bone mineralisation of the birds, or their performance, the FEEDAP Panel concludes that the additive has the potential to be efficacious at the minimum recommended dose (250 FTU/kg feed). These conclusions can be extended to chickens reared for laying and turkeys reared for breeding. Since the mode of action of the phytase is well known and can be considered to be similar in all poultry species, the conclusions drawn for chickens and turkeys for fattening can be extrapolated to minor poultry species for fattening or reared for laying/breeding. Based on the results of the efficacy trials provided in piglets, pigs for fattening and sows, in which the additive significantly improved phosphorus digestibility, phosphorus retention or performance parameters, the FEEDAP Panel concludes that the additive has the potential to be efficacious at 500 FTU/kg in weaned piglets and at 250 FTU/kg in pigs for fattening and sows.

The use of the additive allows the use of diets with a lower level of inorganic phosphorus, which may in turn reduce the excretion of phosphorus.

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BACKGROUND

Regulation (EC) No 1831/2003[5] 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 the company Roal Oy[6] for authorisation of the product Quantum® Blue, 6-phytase, when used as a feed additive for chickens for fattening/reared for laying, turkeys for fattening/reared for breeding, minor poultry species for fattening and breeding, piglets (weaned), pigs for fattening and sows (category: zootechnical additive; functional group: substances which favourably affect the environment) 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.[7] 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 8 October 2012.

The additive Quantum® Blue is a preparation of 6-phytase produced by a genetically modified strain of *Trichoderma reesei* (CBS 126897). This product 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 animal(s), consumer, user and the environment and the efficacy of the product Quantum® Blue (6-phytase), when used under the conditions described in Table 1.

[5] 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.
[6] Roal Oy, Tykkimäentie 15, 05200 Rajamäki, Finland.
[7] EFSA Dossier reference: FAD-2012-0015.

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

Additive	6-phytase EC 3.1.3.26
Registration number/EC No/No (if appropriate)	
Category(-ies) of additive	Zootechnical additive
Functional group(s) of additive	Substances which favourably affect the environment

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Preparation of 6-phytase produced by Trichoderman reesei (CBS 126897) having a minimum activity of: Solid forms: 5 000 FTU/g, 40 000 FTU/g Liquid forms: 5 000 FTU/g, 10 000 FTU/g	-	Complies with the chemical and microbiological purity criteria set by JECFA and FCC	1 FTU is the amount of enzyme which liberates 1 micromole of inorganic phosphate from sodium phytate per minute at 5.5 and 37 C

Trade name (if appropriate)	Quantum Blue 5 L (liquid form) Quantum Blue 10 L (liquid form) Quantum Blue 5 G (solid, granulated form) Quantum Blue 40 P (solid form
Name of the holder of authorisation (if appropriate)	ROAL Oy

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		Units/kg of complete feedingstuffs		
Chickens for fattening	-	250 FTU	-	-
Chickens reared for laying	-	250 FTU	-	-
Turkeys for fattening	-	250 FTU	-	-
Turkeys reared for breeding	-	250 FTU	-	-
Minor poultry species for fattening and breeding	-	250 FTU	-	-
Piglets	-	250 FTU	-	-
Pigs for fattening	-	250 FTU	-	-

Sows		250 FTU		

Other provisions and additional requirements for the labeling	
Specific conditions or restrictions for use (if appropriate)	For use in compound feed rich in phytates
Specific conditions or restrictions for handling (if appropriate)	Indicate the storage temperature, shelf life R42 potential respiratory sensitizer
Post-market monitoring (if appropriate)	Traceability / register of complaints
Specific conditions for use in complementary feedingstuffs (if appropriate)	Recommended dosages per kg of complete feedingstuffs: Chickens for fattening: 250 – 2 500 FTU/kg Chickens reared for laying: 250 – 2 500 FTU/kg Turkeys for fattening: 250 – 2 500 FTU/kg Turkeys reared for breeding: 250 – 2 500 FTU/kg Minor poultry species for fattening and breeding: 250 – 2 500 FTU/kg Piglets: 250 – 1 750 FTU/kg Pigs for fattening: 250 – 1 750 FTU/kg Sows: 250 – 1 750 FTU/kg

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
-	-	-	-

ASSESSMENT

1. Introduction

The additive Quantum® Blue is a preparation of 6-phytase (phytase; EC 3.1.3.26) produced by a genetically modified strain of *Trichoderma reesei* (CBS 126897) that is available in solid (Quantum® Blue 5 G and Quantum® Blue 40 P) and liquid forms (Quantum® Blue 5 L and Quantum® Blue 10 L). The use of 6-phytase in feed should permit to improve assimilation of phosphorus and other minerals. This product has not been previously authorised in the European Union and is intended for use as a zootechnical additive (substances which favourably affect the environment) in chickens for fattening and reared for laying, turkeys for fattening and reared for breeding, minor poultry species for fattening and breeding, piglets (weaned), pigs for fattening and sows.

2. Characterisation[8]

2.1. Characterisation of the additive

The additive is a preparation with phytase as the declared activity, but it also contains other side activities, such as beta-xylanase and beta-glucanase activity.

Quantum® Blue 5 G and Quantum® Blue 40 P are solid forms of the additive that contain a minimum phytase activity of 5 000 FTU[9]/g and 40 000 FTU/g, respectively. Quantum® Blue 5 G is a granular formulation that contains fermentation product (2.7–3.3 %), vegetable oil (7 %), sorbitol (3.5 %), sodium chloride (0.7 %)[10] and wheat flour (to 100 %); Quantum® Blue 40 P is a powdery formulation that contains fermentation product (19.4–24.9 %), vegetable oil (0.4 %) and wheat flour (to 100 %). A study of the batch-to-batch variation of phytase activity in five batches (measured with the method proposed by the applicant) showed a mean value of 7 106 FTU/g with a coefficient of variation (CV) of 12 % for Quantum® Blue 5 G[11] and a mean value of 49 140 FTU/g (CV 10 %) for Quantum® Blue 40 P.[12] The applicant, upon request, provided the batch-to-batch variation for the solid formulations using the method recommended by the European Union Reference Laboratory (EURL) for official control (EN ISO 30024 method). For Quantum® Blue 5 G, the mean value was 6 176 FTU/g (CV 3.9 %) and for Quantum® Blue 40 P the mean value was 47 391 FTU/g (CV 11 %).[13] Beta-xylanase and beta-glucanase amount to 1 665 xylanase units (BXU)/g and 886 beta-glucanase units (BU)/g in Quantum® Blue 5 G and to 14 950 BXU/g and to 5 186 BU/g, respectively, in Quantum® Blue 40 P.

The study of the particle distribution in three batches of Quantum® Blue 5 G and 40 P showed that the mean particle size is 1 076 and 177 µm, respectively.[14] Particles below 50 µm account for 0.2 % of Quantum® Blue 5 G and 5.5 % of Quantum® Blue 40 P, while particles below 10 µm account for 0 and 0.7 %, respectively.[15] Dusting potential, as measured by the Stauber–Heubach test, was found to be negligible for Quantum® Blue 5 G and 0.018 g/m^3 for Quantum® Blue 40 P.[16]

Quantum® Blue 5 L and Quantum® Blue 10 L are liquid formulations which ensure a minimum phytase activity of 5 000 FTU/g and 10 000 FTU/g, respectively, and which contain fermentation product (2.7–5.8 %), sodium benzoate (0.35 %), sorbitol (20–35 %), sodium chloride (1–5 %)[17] and water (to 100 %). A study of the batch-to-batch variation of phytase activity in five batches of each formulation (measured with the method proposed by the applicant) showed a mean value of

[8] This Section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003'.
[9] One phytase unit (FTU) is the quantity of enzyme that liberates 1 µmol of inorganic phosphate per minute from sodium phytate at 37 ºC, pH 5.5, under standard assay conditions.
[10] Sodium chloride may be replaced by 1 to 5 % sodium sulphate or potassium sulphate or ammonium sulphate.
[11] Technical dossier/Section II/Annex II.4 and Supplementary information March 2013/Annex 7.
[12] Technical dossier/Section II/Annex II.5 and Supplementary information March 2013/Annex 8.
[13] Technical dossier/Supplementary information July 2013/Annex 4.
[14] Technical dossier/Section II/Annex II.10.
[15] Technical dossier/Supplementary information March 2013/Annex 9.
[16] Technical dossier/Supplementary information March 2013/Annex 10.
[17] Sodium chloride may be replaced by 1–5 % sodium sulphate or potassium sulphate or ammonium sulphate.

6 250 FTU/g and 11 669 FTU/g for Quantum® Blue 5 L and 10 L, respectively, with a CV of 10.3 and 15.5 %, respectively. The applicant, upon request, provided the batch-to-batch variation using the method recommended by the EURL for official control. For Quantum® Blue 5 L, the mean value was 5 745 FTU/g (CV 9 %), and for Quantum® Blue 10 L the mean value was 11 406 (CV 11 %).[18] beta-xylanase and beta-glucanase amount to 1 069 BXU/g and 538 BU/g, respectively, in Quantum® Blue 5 L, and to 2 475 BXU/g and 1 017 BU/g, respectively, in Quantum® Blue 10 L.[19] Quantum® Blue 5 L and Quantum® Blue 10 L are brown liquid formulations with a density of 1.12–1.13 kg/L, a pH of 4.4–4.6,[20] a viscosity of 3.1–3.3 mPas and a surface tension of 41–42 mN/m.[21]

Three batches of the fermentation product and five batches of each final formulation[22] were analysed for microbiological parameters including total viable counts (< 1 000 colony-forming units (CFU)/g, one batch 20 000 and another one 3 500), coliforms (< 10 CFU/g in solid formulations and < 1 CFU/g in liquid), yeast and moulds (< 1 000 CFU/g), *Escherichia coli* and *Salmonella* (not detected in 25 g). The same batches were investigated for the content of lead, cadmium, copper, mercury and arsenic (total content including the five < 5.64 mg/kg in 5 G; < 2.5 mg/kg in 40 P; < 1.4 mg/kg in the liquid formulations and < 7.07 mg/kg in the fermentation product), arsenic (< 0.5 mg/kg) and mycotoxins including aflatoxin B_1, B_2, G_1 and G_2 (< 0.05 µg/kg), ochratoxin A (< 2 µg/kg), sterigmatocystin (< 10 µg/kg), zearalenone (< 50 µg/kg), T2-Toxin (< 20 µg/kg), fumonisin B2 (<10 µg/kg) and deoxynivalenol (< 300 µg/kg). Antibacterial activity was found to be absent. Total organic solids content (%) was provided for the same batches.

2.2. Characterisation of the production organism

The phytase present in the additive is produced by a genetically modified strain of *Trichoderma reesei*,, deposited at the Centraalbureau voor Schimmelcultures with the deposition number CBS 126897.[23] The technical dossier contains detailed and sufficient information on the parental and recipient microorganism, the different genetic elements introduced in the production strain, the genetic modification process and the genetic and phenotypic traits introduced.

2.3. Manufacturing process

The 6-phytase is produced by submerged, aerobic pure culture fermentation of *T. reesei* CBS 126897. The enzyme is secreted into the fermentation medium and recovered by a series of filtration and ultrafiltration steps that result in a concentrated enzyme solution. This concentrate is used to prepare the formulations of the additive. Absence of the production strain was demonstrated in 20 mL (liquid products) or 2 g (solid products) of five batches of each final formulation and in three batches of the fermentation product used to formulate the additive.[24]

No recombinant DNA was detected in three batches of the most concentrated solid final product (Quantum® Blue 40 P) and in three batches of the fermentation product before within the limits of detection provided by the applicant.[25]

2.4. Stability and homogeneity

2.4.1. Shelf-life of the additive

The shelf-life of the solid forms of the additive (three batches each) was studied at two different combinations of temperature and relative humidity (25 °C/60 % and 40 °C/75 %).[26] The samples were

[18] Technical dossier/Supplementary information July 2013/Annex 4.
[19] Technical dossier/Section II/Annex II.2 and II.3 and Supplementary information March 2013/Annexes 5 and 6.
[20] Technical dossier/Section II/Annexes II.2 and II.3.
[21] Technical dossier/Section II/Annexes II.8 and II.9.
[22] Technical dossier/Section II/Annexes II.1 to II.5 and supplementary information March 2013/Annexes 3 to 8.
[23] Technical dossier/Section II/Annex II.13.
[24] Technical dossier/Section II/Annexes II.1 to II.5 and II.118 and supplementary information March 2013/Annexes 5 to 8.
[25] Technical Dossier/Section II/Annex II.29.
[26] Technical dossier/Section II/Annex II.062 and Supplementary information March 2013.

kept in open Petri dishes for up to 11 months. Recoveries of Quantum® Blue 5 G kept at 25 °C for 11 months (one batch kept for only 8 months) were close to 70 % and ranged from 21 to 50 % after 1 month at 40 °C. Recovery of Quantum® Blue 40 P kept at 25 °C for 12 months was 54 % (65 % after 6 months, 82 % after 3 months) and 50 % after 1 month at 40 °C.

The shelf-life of the liquid formulations (three batches) was studied at three different temperatures (6, 20–23 and 37 °C).[27] The samples were kept in transparent polypropylene test tubes for up to 12 months. Mean recovery of Quantum® Blue 5 L and Quantum® Blue 10 L after 12 months was 96 and 100 %, respectively, at 6 °C, 97 % in both cases at 20–23 °C and 60 and 56 %, respectively, at 37 °C. At 37 °C, recoveries of Quantum® Blue 5 L and Quantum® Blue 10 L were 95 and 88 %, respectively, after three months and 76 % in both cases after six months.

2.4.2. Stability in premixtures and feed

Three batches of each solid formulation were added to four different types of complete premixtures including premixtures for feed for chickens and turkeys for fattening (without choline chloride), piglets and pigs. The samples were kept in closed plastic bottles.[28] The intended dosage was 250 FTU/g for Quantum® Blue 5 G- and 400 FTU/g for Quantum® Blue 40 P-supplemented premixtures. Samples were kept at room temperature (20–23 °C) for six months. Quantum® Blue 5 G-supplemented premixtures showed no modification of the enzyme activity after six months (except in the pig premixture: 78 %) and the Quantum® Blue 40 P-supplemented premixtures showed mean recoveries of 81–88 %.

A mixture of wheat and soya bean (including also minerals and vitamins) was supplemented with Quantum® Blue 5 G or Quantum® Blue 40 P at 1 000 or 166 mg/kg (one batch each).[29] The mixture was subject to pelleting at temperatures of 65 (40 P only), 75, 80, 85, 90, 95 or 100 °C. Two further batches of Quantum® Blue 5 G were added to broiler feed at ~ 5 800 FTU/kg and of Quantum® Blue 40 P were added at ~ 7 400 FTU/kg feed and the feed pelleted at 70, 75, 80, 85, 90, 95 or 100 °C.[30] Recoveries showed losses below 10 % up to 90 °C for Quantum® Blue 5 G or up to 80–85 °C for Quantum® Blue 40 P. Higher temperatures provoked losses > 20 %.

Two batches of Quantum® Blue 5 G were tested in three different diets (chickens and turkeys for fattening and piglets).[31] The supplementation range was 250–1 000 FTU/kg feed. In all cases, samples of the mash and pelleted diets were stored for three months at room temperature (20–23 °C). Recoveries ranged between 83 and 100 % (mean 91 %) and between 62 and 98 % (mean 86 %) for the mash and pelleted diets, respectively. A third batch was tested when added in mash and pelleted feed for chickens for fattening at 20–23 °C.[32] The supplementation rate was close to 5 000 FTU/kg feed, and recovery after three months was 92 and 98 % for the mash and pelleted diet, respectively.

Two batches of Quantum® Blue 40 P were tested in four different diets (laying hens, pigs for fattening (two diets) and sows).[33] The supplementation range was 250–600 FTU/kg. Except in the case of feed for laying hens, only mash samples of which were used, mash and pelleted samples of each diet were stored for three months at room temperature (20–23 °C). Recoveries ranged between 86 and 110 % (mean 100 %) and between 92 and 107 % (mean 98 %) for the mash and pelleted diets, respectively. A third batch was tested in mash and pelleted broiler feed at (20–23 °C).[34] The supplementation rate was ~ 7 000 FTU/kg feed and recovery after three months was 98–100 %.

[27] Technical dossier/Section II/Annex II.061 and Supplementary information March 2013.
[28] Technical dossier/Section II/Annex II.65 to II.68 and Supplementary information March 2013.
[29] Technical dossier/Section II/Annex II.63 and II.64 and Supplementary information March 2013.
[30] Technical dossier/Supplementary information March 2013/Annexes 25 and 26.
[31] Technical dossier/Section II/Annexes II.069, II.071 and II.073 and Supplementary information March 2013.
[32] Technical dossier/ Supplementary information March 2013/Annex 27.
[33] Technical dossier/Section II/Annexes II.72, II.075 and II.076 and Supplementary information March 2013.
[34] Technical dossier/ Supplementary information March 2013/Annex 28.

It is recommended that the liquid formulations be used when the pelleting temperature exceeds 80 °C/90 °C.One batch of the Quantum® Blue 10 L was sprayed onto two pelleted diets, one for chickens for fattening and one for piglets.[35] In each case, three supplementation levels were tested, ranging from 170 to 1 000 FTU/kg feed. Samples were kept for three months at room temperature (20–23 °C). Recoveries ranged between 62 and 85 % (mean value 75 %). One batch of Quantum® Blue 5 L was added to pelleted starter and grower feed for chickens for fattening at a dose of 1 500 FTU/kg feed and the feed stored for three months at 20–23 °C.[36] After three months, 99–102 % of the activity was recovered. A third batch was added at a dose of 700 FTU/kg to feed for chickens for fattening pelleted at 82 °C and the feed stored for three months at 20–23 °C.[37] Recovery was 105 % after three months.

2.4.3. Homogeneity

The capacity of the solid preparation of the additive to homogeneously distribute was studied in premixtures and feed; distribution of the liquid preparation was studied in pelleted feed only.

Quantum® Blue 5 G and 40 P were mixed in complete premixtures for poultry at a dose of ~ 60 FTU/g.[38] Twenty samples of the premixture were analysed; the mean value was 57 and 62 FTU/g for Quantum® Blue 5 G and 40 P, respectively, and the corresponding CVs were 17 and 6 %.

Quantum® Blue 5 G and 40 P were mixed in a feed for poultry at an intended dose of ~ 500 FTU/kg.[39] Twenty samples of the feed were analysed; the mean value was 718 and 617 FTU/kg for Quantum® Blue 5 G and 40 P, respectively, and the corresponding CVs were 18 and 5 %.

Quantum® Blue 10 L was sprayed onto two pelleted feeds (one for poultry and one for piglets) at an intended dose of ~ 500 FTU/kg.[40] Twenty samples of the feed were analysed; the mean value was 339 and 319 FTU/kg, respectively, and the corresponding CVs were 11 and 16 %.

2.5. Conditions of use

The additive is to be used in feed for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, minor poultry species for fattening and breeding, piglets, pigs for fattening and sows at a minimum dose of 250 FTU/kg. The maximum recommended dose is 2 500 FTU/kg feed for poultry and 1 750 FTU/kg feed for pigs.

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 Appendix.

The method proposed by the EURL to control the phytase content of the additive is different to the one proposed by the applicant. The applicant provided, on request, data showing that the formulations comply with the specifications of the additive when phytase activity is measured using the method proposed by EURL (see section 2.1).

Similarly, the method used to determine the phytase content of experimental feeds was the in-house method proposed by the applicant to measure the phytase. This method measures phytase in quantum phytase units (QPU). The values are transformed into FTU (ISO method) by a conversion factor. Use of this factor was supported by analytical data obtained in different types of feeds and formulations.

[35] Technical dossier/Section II/Annexes II.070 and II.074 and Supplementary information March 2013.
[36] Technical dossier/ Supplementary information March 2013/Annex 29.
[37] Technical dossier/ Supplementary information March 2013/Annex 30.
[38] Technical dossier/Section II/Annexes II.78 and II.79.
[39] Technical dossier/Section II/Annexes II.80 and II.81.
[40] Technical dossier/Section II/Annexes II.82 and II.83.

3. Safety[41]

3.1. Safety aspects of the genetic modification

Neither the recombinant DNA nor the introduced phenotypic traits give rise to any safety concerns. The applicant provided sufficient information that the production strain and recombinant genes were not detected in the final product.

3.2. Safety for the target species

3.2.1. Safety for chickens for fattening

A total of 750 one-day-old male chickens (Ross 308) were grouped in groups of 10 birds and allocated to one of five dietary treatments (15 replicates per treatment).[42] Starter and grower basal diets based on maize and soya bean meal and with a total phosphorus content of 0.64 % (starter) or 0.50 % (grower) were supplemented with phytase concentrate to provide 0, 500, 2 500 (1×) or 250 000 (100×) FTU/kg feed (confirmed by analysis). A diet with a total phosphorus content of 0.75 % (starter) or 0.65 % (grower) was also included in the experiment. Diets were offered in pelleted form for 35 days. Feed intake, body weight and mortality were recorded and feed to gain ratio was calculated. Analysis of variance (ANOVA) was performed with the data and group means were compared using the Tukey test.

Mortality was within the normal limits and was not treatment related (2, 3, 3, 5 and 2 % in the five groups). Daily feed intake (g/day) of the birds was 99, 103, 106, 105 and 103 in the 0, 500, 2 500, 250 000 FTU/kg and positive control group, respectively; final body weight (kg) was 2.5, 2.6, 2.7, 2.7, 2.6, respectively and feed to gain ratio was 1.41, 1.39, 1.39, 1.38, 1.42, respectively. Supplementation of the diet with the phytase resulted, compared to the 0 FTU/kg diet, in a higher daily feed to gain ratio (all dosages), higher body weight (all dosages) and a better feed to gain ratio (2 500 FTU/kg group). Therefore, the supplementation of the experimental diets with the phytase contained in Quantum® Blue at up to 100× the maximum recommended dose did not have a negative effect on the performance of the birds.

3.2.2. Safety for turkeys for fattening

A total of 288 day-old female turkeys (BUT Big 6) were housed in pens with nine birds per pen (eight replicates per treatment).[43] During the study, three basal diets were used according to the birds' requirements (phase 1, 2 and 3 diets). The three basal diets were based on maize, wheat and soya bean meal with a total phosphorus content of 0.75 (phase 1), 0.69 (phase 2) or 0.61 % (phase 3) and were supplemented with phytase concentrate to provide 0, 1 000, 2 500 (1×) or 250 000 (100×) FTU/kg feed (confirmed by analysis). Diets were offered in pelleted form for 63 days. Feed intake, body weight and mortality were recorded and feed to gain ratio was calculated. One bird per pen was slaughtered at the end of the experiment and blood samples were collected and analysed for alanine transaminase, glutamate dehydrogenase, alkaline phosphatase, urea, proteins, lipase, calcium and phosphorus. A one-way ANOVA was performed with the data and group means were compared using the Tukey test.

Four animals died, two in the control group and one each in the 2 500 FTU/kg and 250 000 FTU/kg groups. Daily feed intake (g/day) was 137, 144, 130 and 140 in the 0, 1 000, 2 500 and 250 000 FTU/kg group, respectively; final body weight was 5.0, 5.2, 4.8, 5.2 kg, respectively, and feed to gain ratio was 1.74, 1.75, 1.72, 1.72, respectively. Some statistically significant differences in the performance of the birds were found between the 2 500 FTU/kg group and the other phytase-supplemented groups (feed intake was significantly lower in the 2 500 FTU/kg group than in the 1 000 FTU/kg group and final body weight in the 2 500 FTU/kg group was significantly lower than in

[41] This Section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003
[42] Technical dossier/Section III/Annexes III.1 to III.3.
[43] Technical dossier/Supplementary information March 2013/Annexes 31 to 35.

the other two supplemented groups). However, no differences between the performance of the birds in the phytase-supplemented groups and the non-supplemented group were reported. Blood parameters did not differ in the different dietary groups. Therefore, supplementation of the experimental diets with the phytase contained in Quantum® Blue at up to 100× the maximum recommended dose did not have a negative effect on the performance of the birds.

3.2.3. Safety for piglets

A total of 480 male and female piglets (commercial breed) were distributed in pens of 12 piglets each (10 replicate pens per treatment) distributed in a total of five rooms (eight pens each).[44] The animals entered in the study in five consecutive weeks; each group of animals was placed in one room and pens were allocated to one of the dietary treatments (two pens per treatment each week). Piglets were 26 days old and the average body weight was 8.5 kg. Weaner and starter diets based on barley, wheat and soya bean meal and with a total phosphorus content of 0.58 % (weaner) and 0.50 % (starter) were supplemented with phytase to provide 0, 2 500 or 250 000 FTU/kg feed. Enzyme activity was confirmed in the 2 500 FTU/kg diet, but recoveries showed that the intended dosage in the 250 000 FTU/kg diet was ~ 175 000 FTU/kg.[45] A fourth experimental treatment considered a diet with total phosphorus content of 0.69 % (weaner) and 0.65 % (starter). Diets were offered as mash for 42 days. Mortality, feed intake and body weight were recorded and feed to gain ratio calculated. ANOVA was performed with the data and group means were compared using the Bonferroni test.

Five piglets died (one in the 0 FTU/kg diet group, two in the 250 000 FTU/kg group and one in the positive control group). No significant differences in the performance of the piglets were observed. Daily feed intake ranged from 803 to 821 g, mean final body weight was 31 kg, daily weight gain ranged from 526 to 550 g and feed to gain ratio ranged from 1.49 to 1.53. The supplementation of the experimental diets with the phytase contained in Quantum® Blue up to 175 000 FTU/kg feed did not have a negative effect on the performance of the piglets.

3.2.4. Safety for pigs for fattening

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

3.2.5. Safety for sows

A total of 46 sows (Large White × Landrace) were housed in groups of 9 or 10 animals after insemination and until five days before the expected date of farrowing, when they were transferred to individual farrowing pens.[46] The groups of sows were assigned to one of the dietary treatments. The study lasted until weaning of the piglets (day 33 of life). Gestation and lactation diets, based on maize, barley and soya bean meal with a total phosphorus content of 0.44 % (gestation diet) or 0.48 % (lactation diet) were supplemented with phytase concentrate to provide 0, 250, 2 500 or 250 000 FTU/kg feed. Enzyme recoveries in the experimental diets were lower than intended in the 2 500 and 250 000 FTU/kg diets, reaching values around 1 750 and 195 000 FTU/kg feed, respectively.[47] Positive control diets with a total phosphorus content of 0.63 % (gestation diet) or 0.67 % in (lactation diet) were also included. Feed was offered in mash form (restricted during gestation). ANOVA was performed with the data and group means were compared using the least significant difference (LSD) test.

Sows were weighed at insemination, before moving them to the farrowing sites, and at weaning. Feed intake was measured per group during pregnancy and individually during lactation. Farrowing and lactation performance were measured as follows: number of pigs born alive and stillborn, number

[44] Technical dossier/Section III/Annexes III.11 to II.14 and Supplementary information March 2013/Annex 36.
[45] The recommended maximum dose was initially set at 2 500 FTU/kg. However, owing to the low recovery in the experimental diets, the applicant changed the recommendation to 1 750 FTU/kg.
[46] Technical dossier/Section III/Annexes III.15 to III.18 and Supplementary information March 2013/Annexes 64 and 65.
[47] The recommended maximum dose was initially set at 2 500 FTU/kg However, owing to the low recovery in the experimental diets, the applicant changed the recommendation to 1 750 FTU/kg.

alive at 21 days and at weaning and litter weight at day 1, day 21 and at weaning. Piglets had no access to creep feed. Average parity per group was reported. Digestibility of the diets was measured during the study (data reported section 4.5, Efficacy for sows).

No differences between the dietary treatments were found in any of the parameters evaluated (digestibility not included) with the exception of the sows' feed intake during lactation, with feed intake being higher in the sows receiving the positive control feed than in those in the other dietary groups. At farrowing, mean litter size (live piglets) was 10.7 and mean litter weight was 14.5 kg, and at weaning mean litter size was 9.5 piglets and mean litter weight was 79 kg. Supplementation of the experimental diets with phytase contained in Quantum® Blue at doses up to 195 000 FTU/kg feed did not have a negative effect on the performance of the sows.

3.2.5.1. Conclusions on the safety for species

The results of the tolerance trials in chickens and turkeys for fattening, weaned piglets and sows showed that the animals tolerated well 100-fold the maximum recommended dosage (2 500 FTU/kg for poultry and 1 750 FTU/kg for pigs). Therefore, the FEEDAP Panel concludes that the additive is safe for these target species at the corresponding maximum recommended dose. The conclusions can be extended to chickens reared for laying, turkeys reared for breeding (maximum dose of 2 500 FTU/kg feed) and pigs for fattening (maximum dose of 1 750 FTU/kg feed)

Considering the margin of safety demonstrated in major poultry species, the conclusions on the safety for those species can be extrapolated to minor poultry species for fattening or reared for laying/breeding, provided that the same maximum dose applies.

3.3. Safety for the consumer

The studies provided under this section were performed with a mixture of three spray-dried batches of the fermentation product (not containing stabilising agents or preservatives) used to formulate Quantum® Blue with a phytase activity ~ 227 000 FTU/g.[48]

3.3.1. Genotoxicity studies including mutagenicity

3.3.1.1. Bacterial reverse mutation assay

The mutagenic potential of the fermentation product (suspended in deionised water) was tested in *Salmonella* Typhimurium strains TA1535, TA1537, TA98 and TA100 and in *Escherichia coli* strain WP2 uvrA in the presence and in the absence of metabolic activation (S9 fraction from liver of rats pre-treated with phenobarbital/β-naphthoflavone) up to a concentration of 5 000 µg per plate, in compliance with OECD Guideline 471.[49] The results for revertant colony numbers suggest that the test item is not mutagenic. Appropriate reference mutagens used as positive controls showed a distinct increase in induced revertant colonies.

3.3.1.2. Chromosome aberration test

The fermentation product (suspended in deionised water) was assessed for its potential to induce structural chromosome aberrations in V79 cells *in vitro* in two independent experiments in the absence and presence of metabolic activation (S9 fraction from liver of rats pre-treated with phenobarbital/β-naphthoflavone).[50] In each experimental group, two parallel cultures were set up. At least 100 metaphases per culture were evaluated for structural chromosome aberrations up to a concentration of 5 000 µg/mL. In the absence of S9 mix, no clastogenicity was observed at the concentrations evaluated in any experiment both in the absence and in the presence of S9 mix. No increase in polyploid metaphases was noticed after treatment with the test item compared with the control cultures. Appropriate mutagens used as positive controls induced statistically significant increases in

[48] Technical dossier/Section II/Annex II.1.
[49] Technical dossier/Section III/Annex III.19.
[50] Technical dossier/Section III/Annex III.20.

the number of cells with structural chromosome aberrations. Only 100 metaphases per culture were evaluated for structural chromosome aberrations, rather than at least 200, as recommended by OECD Guideline 473. In spite of this minor deviation from OECD Guideline, the study provides evidence that the test item is not clastogenic.

3.3.1.3. *In vivo* micronucleus test

The potential of the fermentation product to induce micronuclei in polychromatic erythrocytes (PCEs) from bone marrow was tested *in vivo* in Wistar rats up to a dose level of 2 000 mg/kg bw (oral administration) in accordance with OECD Guideline 474.[51] The test item was suspended in 0.9 % saline, which was also used as vehicle control. The bone marrow cells were collected for micronuclei analysis 24 and 48 hours after a single administration of the test item. Seven males per test group were evaluated for the occurrence of micronuclei and 2 000 PCEs per animal were scored for micronuclei.

No biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei was observed at any sampling time and any dose level used, while the positive control showed an increase in induced micronucleus frequency. The test item did not exert any cytotoxic effects in the bone marrow as detected by the alteration of the ratio between polychromatic and normochromatic erythrocytes. The results of this study do not show evidence of mutagenicity although there was no evidence of target tissue exposure.

3.3.2. Subchronic oral toxicity study

The systemic toxicity of the fermentation product was investigated in a subchronic toxicity study in SPF-bred Wistar rats, in compliance with OECD Guideline 408.[52] The test item was administered daily by oral gavage to animals of both sexes at dose levels of 100, 300 and 1 000 mg/kg bw per day for a period of 13 weeks. A control group was treated with the vehicle, bidistilled water, only.

Clinical signs, outside cage observation, food consumption and body weights were recorded periodically during acclimatisation and the treatment periods. Functional observational battery, locomotor activity and grip strength were measured during week 13. At the end of the dosing period, blood samples were withdrawn for haematology and plasma chemistry analyses. Urine samples were collected for urinalyses. All surviving animals were killed and necropsy was performed. Histological examinations were performed on organs and tissues from all control and high-dose animals, and on all gross lesions from all animals.

The death on day 13 of a male treated with 1 000 mg/kg bw per day was not considered a treatment-related effect. No evident clinical signs were reported during daily or weekly observation performed from week 1 to week 12 of treatment, or during the functional observational battery performed during week 13 of treatment, at any dose level. The mean fore- and hindlimb grip strength of the test item-treated males and females were similar to those of the corresponding controls. Minor sporadic differences in the mean locomotor activity of the test item-treated rats were ascribed to typical biological variation and not to systemic toxicity. No test item-related effects were noted in the eyes of the rats at any dose level. The mean daily food consumption of the test item-treated males and females were similar to that of the corresponding controls. There were no treatment-related adverse effects on body weight gain. There were no test item-related changes in the haematology parameters at any dose level. Slightly elevated plasma sodium levels noted in the males and females treated with 1 000 mg/kg bw per day were considered to be test item related, but in the absence of other adverse effects this result was not regarded as toxicologically relevant. There were no test item-related changes in the remaining clinical biochemistry parameters at any dose level. The urinalysis parameters of the test item-treated rats were considered to be unaffected at all dose levels. There were no test item-related changes in the mean absolute or relative organ weights in males or females. No gross lesions could be

[51] Technical dossier/Section III/Annex III.21.
[52] Technical dossier/Section III/Annex III.22.

attributed to treatment with the test item and no histological evidence of toxicity was observed in the organs and tissues examined.

3.3.3. Conclusions on the safety for the consumer

The fermentation product that is used to prepare the final formulations of Quantum® Blue tested negative in genotoxicity tests. The results obtained in a subchronic oral toxicity study raised no concerns regarding the product. Therefore, based on the toxicological studies performed with the fermentation product, the additive is of no concern regarding consumer safety.

3.4. Safety for the user

3.4.1. Skin and eye irritation studies

The applicant provided the results of a skin irritation study of three formulations of Quantum® Blue: 5 G, 40 P and 10 L.[53] The tests followed OECD Guideline 404. In each study, a sample of 0.5 g/mL of the test item was applied by topical semi-occlusive application for four hours to three young adult New Zealand White rabbits. The scoring of skin reactions was carried out after the removal of the dressing at 1, 24, 48 and 72 hours (and up to seven days for Quantum® Blue 40 P). The test items did not induce significant or irreversible damage to the skin. Therefore, the test items are considered to be non-irritant to the skin.

The applicant provided the results of a eye irritation study of three formulations of Quantum® Blue: 5 G, 40 P and 10 L. The items were assayed in Himalayan rabbits, in accordance with OECD Guideline 405.[54] For each test item, three young adult males were treated with a single ocular instillation of 100 mg or 0.1 mL of material in the conjunctival sac of the right eye. Conjunctival redness (grade 1) and chemosis (grade 1) were observed in all animals 1 hour after instillation and in some animals until 24 hours after instillation but in no animals after 48 hours. The corneas and irises were not affected by the treatment. There were no systemic intolerance reactions. Therefore, the test items are considered as non-irritant to the eyes.

3.4.2. Skin sensitisation studies

The possible contact allergenic potential of Quantum® Blue was assessed for three formulations: 5 G, 40 P and 10 L.[55] A local lymph node assay was conducted in CBA/CaOlaHsd mice, in compliance with OECD Guideline 429. The highest concentration tested was the highest concentration that could be achieved whilst avoiding systemic toxicity and excessive local skin irritation as confirmed by a pre-experiment. The results showed that the test items Quantum® Blue 5 G, 40 P and 10 L were not skin sensitisers under the experimental conditions used.

3.4.3. Conclusions on the safety for the user

Three final formulations, Quantum® Blue 5 G, 40 P and 10 L, were tested for skin and eye irritation and skin sensitisation. The results revealed no evidence of dermal or eye irritation or skin sensitisation. The other final liquid formulation, Quantum® Blue 5 L, was not tested, but based on its composition its irritant potential or skin sensitisation potential is unlikely to be significantly different from that of its tested counterpart (Quantum® Blue 10 L). Taking account of the proteinaceous nature of its active substance, the additive is to be 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 raise any environmental safety concern associated with the genetic modification.

[53] Technical dossier/Section III/Annexes III.23 to III.25.
[54] Technical dossier/Supplementary information March 2013/Annexes 37 to 39.
[55] Technical dossier/Section III/Annexes III.26 to III.28.

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

The FEEDAP Panel recognises that some of the studies provided to support the efficacy of the additive in piglets and pigs for fattening do not comply with the requirements established in the latest guidance for zootechnical additives (EFSA FEEDAP Panel, 2012). However, considering that this application was submitted shortly after the publication of this guidance document, the Panel will exceptionally consider such studies in the assessment of the efficacy of the additive.

4.1. Efficacy for chickens for fattening

Trial 1

A digestibility and bone mineralisation study was performed with 300 one-day-old male chickens (Ross 308) that were initially allocated in cages of five animals to five dietary treatments (12 replicates per treatment).[56] On day 4, the number of birds per cage was reduced to three. A basal diet based on maize, barley and soybean meal with a total phosphorus content of 0.32 % was supplemented with Quantum® Blue 40 P to provide 0, 250, 500 or 750 FTU/kg feed (confirmed by analysis). A positive control was also included (total phosphorus content 0.61 %). Feed contained titanium dioxide as an external marker and was offered *ad libitum* from day 1 to day 22 of life. During the first week, the feed was provided in mash form and thereafter in pelleted form. Performance of the birds was monitored throughout the study. On day 22, all the birds were killed and the ileal contents of birds in each cage were pooled. Ileal samples were analysed to calculate phosphorus and calcium apparent digestibility. The tibia from one animal per pen was collected for mineral content analysis. Tibia ash, phosphorus and calcium content was measured. A two-way ANOVA was performed with the data and the comparison of the means was performed using the Duncan test. Results relevant for the assessment are presented in Table 2.

Table 2: Effect of Quantum® Blue on the ileal digestibility of phosphorus and calcium and on the tibia ash and phosphorus content of chickens for fattening

Intended dose (FTU/kg)	Mortality (%)	Ileal digestibility (%)		Tibia content (%, dry matter basis)	
		P	Ca	Ash	P
0	15.0^{a}	49.0^{c}	49.2^{b}	38.4^{d}	6.0^{c}
250	1.7^{b}	57.4^{b}	56.9^{a}	41.2^{c}	6. 8^{b}
500	0^{b}	67.4^{a}	54.2^{a}	42.6^{bc}	7.1^{ab}
750	0^{b}	67.5^{a}	51.5^{a}	43.9^{b}	7.4^{a}
Positive control	1.7^{b}	49.0^{c}	35.8^{c}	46.0^{a}	7.5^{a}

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

Mortality was low, except in the negative control group (positive control, 1.7 %; negative control, 15 %; 250 FTU/kg, 1.7 %; 500 FTU/kg 0 %; and 750 FTU/kg 0 %). Phytase supplementation with Quantum® Blue resulted in a significant improvement in performance parameters compared with birds fed the 0 FTU/kg diet (data not shown). Quantum® Blue at a dose of 250 FTU/kg and above had significant positive effects on digestibility and on bone measurements compared with the 0 FTU/kg group.

Trial 2

[56] Technical dossier/Section IV/Annex IV.1 to IV.3 and supplementary information March 2013/Annex 40.

Two studies, a balance study and a digestibility study, including bone mineralisation, were reported in this trial.[57] The chickens were Ross 308 and the same diets were used in each study. Two basal diets based on maize and soya bean meal with different calcium content (0.98 % and 1.30 %, respectively) with a total phosphorus content of 0.51 % were supplemented with Quantum® Blue to provide 0, 250, 500 and 750 FTU/kg (confirmed by analysis). Diets were offered as pellets and contained titanium dioxide as an external marker.

The balance trial was conducted in two runs, one starting on day 18 of life and the other one on day 25. Before the start of the balance study, the birds were fed a commercial starter diet with an adequate content of phosphorus and calcium. In each run, a total of 40 birds were distributed to one of the eight treatments (n = 5 birds per treatment per run). After three days of adaptation to their experimental diet, birds were individually caged and fed the same diet for a further nine days. Total excreta was collected for five days starting on day 5 (during this period feeding was restricted).

The digestibility and bone mineralisation study was performed with a total of 880 birds distributed in 80 pens (11 birds per pen, 10 pens per treatment). On day 21 of life, the groups of birds were allocated to the different dietary treatments (allocation ensured equal distribution of the treatments in the house). Dietary treatments were offered *ad libitum* for nine days. After this period, nine birds (30 days old) per pen were sacrificed and ileum digesta samples were collected and pooled per pen (n = 10). The remaining birds continued under study until day 35 of life (14 days under treatment), when two birds per pen (n = 20) were sacrificed to collect the left tibia bone.

Digesta and excreta samples were analysed to determine phosphorus and calcium content and tibia bone was analysed for ash, phosphorus and calcium content. The two groups receiving the dietary treatment containing 500 FTU/kg were in the end not considered because the analytical content of calcium in the two diets was similar. ANOVA was performed with the data and the effect of phytase supplementation compared with control was tested by the Dunnett test. For the balance trial, the two runs were analysed separately. Results relevant for the assessment are presented in Table 3.

Table 3: Effect of Quantum® Blue on the ileal digestibility and utilisation of phosphorus and calcium and bone mineralisation of chickens for fattening

Intended dose (FTU/kg)	Calcium	Ileal digestibility (%)		Utilisation (%) [1]		Tibia content (%)	
		P	Ca	P	Ca	Ash [2]	P [3]
0	+	35.8	37.3	37/38	16/25	42.7	18.2
250	+	51.1*	45.9*	52*/49*	42*/37*	44.9*	18.3
750	+	50.6*	29.8	45/44	33*/25	45.8*	18.7*
0	–	43.8	45.8	46/39	30/32	42.3	18.7
250	–	54.7*	46.8	56*/48*	52*/50*	45.1*	18.8
750	–	55.9*	36.3*	42/40	41/33	47.2*	19.0*

* Values are different from the corresponding control group, Dunnett test.
1 Values are for the first/second run, respectively.
2 Values are expressed on dry matter basis.
3 Values are expressed as phosphorus content (per cent) in ash.

No animals died during the studies and the performance of the animals from day 21 to 30 did not differ between groups (data not shown). Compared with the 0 FTU/kg diet, phytase supplementation at 250 FTU/kg resulted in an increase in ileal digestibility of phosphorus, phosphorus utilisation and tibia ash content, regardless of the calcium content of the diet. Compared with the 0 FTU/kg diet, phytase

[57] Technical dossier/Section IV/Annex IV.4 to IV.6 and supplementary information March 2013/Annex 41.

supplementation at 750 FTU/kg resulted in a significant increase in, ileal digestibility of phosphorus, tibia ash content and phosphorus content in tibia bone regardless of the calcium content of the diet.

Trial 3

A combined performance, digestibility and bone mineralisation trial was performed.[58] For the performance study, a total of 1 280 one-day-old male chickens (Ross 308) were distributed in 40 pens of 32 birds and allocated to four dietary treatments (10 replicates per treatment). Starter and grower diets based on maize and soya bean meal with a total phosphorus content of 0.46 % (starter) or 0.42 % (grower) were supplemented with Quantum® Blue 40 P to provide 0, 250 or 500 FTU/kg feed (confirmed by analysis). A positive control was also considered (total phosphorus content 0.67 % (starter) and 0.66 % (grower)). Feed was offered *ad libitum* from day 1 to day 35 of life in coarse meal form (starter) or pelleted form (grower). Performance of the birds was monitored throughout the study. On day 36 of the study, 20 birds per treatment were fasted for one day and slaughtered to measure carcass parameters (data not shown). At the end of the performance trial, a digestibility study was performed with 10 birds (34 days old) per treatment, which were individually caged and were offered the same feed they ate previously. Birds received the diets for 10 days, and in the last three days the diets contained titanium dioxide as an external marker. On day 10 of the digestibility study, birds were killed, ileal samples were collected and phosphorus and calcium were determined. Tibia bones were collected in order to determine ash, phosphorus and calcium content. ANOVA was performed with the data and the comparison of the means was done with the Tukey test. The results are presented in Table 4.

Table 4: Effect of Quantum® Blue on the performance, ileal digestibility of phosphorus and calcium and bone mineralisation of chickens for fattening

Intended dose (FTU/kg)	Daily feed intake (g)	Final body weight (g)	Feed to gain ratio	Mortality (%)	Ileal digestibility (%)		Tibia content (%)	
					P	Ca	Ash [1]	P [2]
0	77[b]	1 814[b]	1.58[a]	8.8	40.6[c]	62.5[a]	19.5[b]	8.2[c]
250	90[a]	2 166[a]	1.50[b]	3.8	47.6[b]	57.2[b]	24.1[a]	9.3[b]
500	90[a]	2 144[a]	1.52[b]	5.6	51.7[a]	58.1[b]	24.9[a]	9.2[b]
Positive control	90[a]	2 193[a]	1.53[ab]	5.3	48.5[b]	40.2[c]	25.4[a]	9.7[a]

[a,b,c] Values within one column with different superscripts are significantly different ($P < 0.05$).
[1] Values are on fresh matter basis.
[2] Values are expressed as phosphorus content (per cent) in bone dry matter.

Performance of the birds, digestibility of phosphorus and bone mineralisation was improved with Quantum® Blue phytase supplementation from 250 FTU/kg feed.

The results from the three trials in chickens for fattening showed that the supplementation of feed with Quantum® Blue at the minimum recommended dose of 250 FTU/kg resulted in a significant increase in ileal phosphorus and tibia ash/phosphorus content in all three trials as well as in phosphorus utilisation in trial 2 and in performance of the birds in trial 3. Therefore, the additive has the potential to improve the performance and phosphorus utilisation/retention in chickens for fattening.

[58] Technical dossier/Section IV/Annex IV.7 to IV.10 and supplementary information March 2013/Annex 42.

4.2. Efficacy for turkeys for fattening

Trial 1

A balance trial was performed with 40 female 28-day-old turkeys (BUT Big 6; 1 109 g bw) that were individually housed in balance cages and allocated to four dietary treatments (10 replicates per treatment).[59] A basal diet based on maize and soya bean meal with a total phosphorus content of 0.52 % was supplemented with Quantum® Blue 5 G to provide 0, 250, 500 or 1 000 FTU/kg feed (the results showed that values were lower than intended). Feed was offered on restricted basis (100 g/day) and as pellets. The experimental period lasted for 10 days, comprising a period of six days of adaptation and four days of excreta collection (once daily). Birds were weighed at the beginning and at the end of the study. Excreta samples were analysed to study organic matter, phosphorus and calcium utilisation. ANOVA was performed with the data and the comparison of the means was done with the Dunnett and Tukey tests.

Feed intake, body weight and feed conversion ratio did not differ between treatment groups (data not shown). The results showed that phytase supplementation with Quantum® Blue from 250 FTU/kg feed resulted in significantly higher phosphorus utilisation compared with no supplementation (phosphorus utilisation 48, 55, 55 and 58 % in the 0, 250, 500 and 1 000 FTU/kg feed group, respectively). Calcium utilisation was significantly higher than in the control only in the 1 000 FTU/kg feed group (49, 50, 50 and 56 % in 0, 250, 500 and 1 000 FTU/kg feed group, respectively).

Trial 2

A balance trial was performed with 40 male and female 22-day-old turkeys (BUT Big 6; 850 g bw) that were individually housed in balance cages and allocated to one of four dietary treatments (10 replicates per treatment).[60] A basal diet based on maize, and soya bean meal with a total phosphorus content of 0.57 % was supplemented with Quantum® Blue 40 P to provide 0, 250, 500 or 750 FTU/kg feed (the results showed that values were lower than intended). Feed, containing titanium dioxide as an external marker, was offered on a restricted basis (80 g/day) in pelleted form. The experimental period lasted for nine days, comprising a period of four days of adaptation and five days of excreta collection. Birds were weighed at the beginning and at the end of balance period. Excreta samples were analysed for phosphorus and calcium utilisation. ANOVA was performed with the data and the means were compared using the Dunnett test.

Feed intake and body weight did not differ between treatment groups (data not shown). The results showed that, compared with the group receiving the non-supplemented diet, phosphorus utilisation (measured with the external marker) was significantly higher in the groups receiving feed supplemented with phytase from 250 FTU/kg feed (45, 52, 58 and 61 % in the 0, 250, 500 and 750 FTU/kg feed group, respectively). The same trend was found for calcium utilisation (5, 15, 20 and 24 % in 0, 250, 500 and 750 FTU/kg feed group, respectively) (in some cases negative values on calcium utilisation were obtained, those were not included in the calculation of mean values). Utilisation as calculated from the quantitative measurements was similar to that measured with the external marker (data not shown).

Trial 3

A combined performance and digestibility study was performed.[61] In the performance study, a total of 768 one-day-old female Hybrid turkeys were penned in groups of 32 birds and allocated to one of four dietary treatments (six pens per treatment). Starter, grower and finisher diets, based on maize and soya bean meal and with a total phosphorus content of 0.71 % (starter), 0.59 % (grower) or 0.58 % (finisher) were supplemented with Quantum® Blue 40 P to provide 0, 250 or 500 FTU/kg feed

[59] Technical dossier/Section IV/Annexes 11 to 13 and supplementary information March 2013/Annex 43.
[60] Technical dossier/Section IV/Annexes 14 to 16 and supplementary information March 2013/Annex 44.
[61] Technical dossier/Section IV/Annexes 17 to 20 and supplementary information March 2013/Annex 45.

(confirmed by analysis). A positive control was also included (total phosphorus content of 1.0, 9.3 and 6.6 % in the starter, grower and finisher diet, respectively). Feed was offered *ad libitum* for 84 days in coarse meal form (until day 15) or as pellets (from day 15 to day 84). Mortality was monitored throughout the study. The birds were weighed, feed intake was measured and feed to gain ratio was calculated. Carcass parameters at slaughter were also determined (data not shown). On day 55, a total of 10 birds per treatment were randomly selected and placed in individual metabolic cages. The birds received the diets they had previously received for seven days, but containing titanium dioxide as an external marker. Birds were killed and ileal samples were collected. Digestibility of dry matter and phosphorus and calcium content were studied. ANOVA was performed with the data and mean values were compared with the Tukey test. The results are presented in Table 5.

Mortality was low, except in the 500 FTU/kg dietary group, in which a large number of birds died for 'technical reasons' at the beginning of the experiment. Therefore, the deaths were not associated with the dietary treatments. The results showed that phytase supplementation with Quantum® Blue significantly increased body weight and phosphorus ileal digestibility compared with no supplementation.

Table 5: Effect of Quantum® Blue on the performance, ileal digestibility of phosphorus and calcium and bone mineralisation of turkeys for fattening

Intended dose (FTU/kg)	Daily feed intake (g)	Final body weight (g)	Feed to gain ratio	Mortality (%)	Ileal digestibility (%)	
					P	Ca
0	192	7 560[b]	2.17	1.6	31[d]	42[b]
250	195	7 792[a]	2.15	5.2	33[c]	39[cb]
500	191	7 829[a]	2.13	9.4	38[b]	49[a]
Positive control	194	7 896[a]	2.17	4.7	44[a]	36[c]

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

The results from the three trials showed that the supplementation of the feed with Quantum® Blue at the minimum recommended dose of 250 FTU/kg resulted in a significant increase in the phosphorus utilisation (trials 1 and 2) and performance of the turkeys (trial 3). Therefore, the additive has the potential to improve performance and phosphorus utilisation in turkeys for fattening.

4.3. Efficacy for weaned piglets

The applicant provided a retention study, two digestibility trials, a performance trial that included digestibility and bone mineralisation and a second performance trial that included bone mineralisation. The retention study[62] and the second performance trial[63] were not considered further in the assessment because the piglets received medication (apramycin sulphate) in their drinking water for seven days prior to the start of the study.

Trial 1

A total of 20 castrated male pigs (Pi × (DE × DL)) were used, starting at six weeks of age.[64] Pigs were kept in pens until they reached 13 kg body weight (fed a commercial feed) and thereafter were allocated to five dietary treatments. A basal diet based on maize and soya bean meal with a total phosphorus content of 0.38 % was supplemented with Quantum® Blue 40 P to provide 0, 250, 500, 750 or 1 000 FTU/kg feed (confirmed by analysis). The diets were offered in pelleted form. The trial was run in two separate collection periods. Dietary treatment received by the pigs was changed

[62] Technical dossier/Section IV/Annexes IV.21 to IV.23 and supplementary information March 2013/Annexes 47 to 49.
[63] Technical dossier/Section IV/Annexes IV.28 to IV.30 and supplementary information March 2013/Annex 47 and 50.
[64] Technical dossier/Section IV/Annex IV.24 to IV.27 and supplementary information March 2013/Annex 46.

between periods. Before the start of the collection periods, the animals had a 10-day period of adaptation to the diets. Two days before the start of the collection period they were moved to the cages. Total faeces were collected for five days. Three piglets had to be withdrawn from study during the first collection period (one each in the control, 250 FTU/kg and 750 FTU/kg groups), resulting in seven replicates for these treatments. During the collection periods, feed allowance was 300 g/day in the first period and 400 g/day in the second. Feed refusals were quantified. Faeces were analysed to determine organic matter, phosphorus and calcium in order to calculate digestibility. ANOVA was performed with the data and means were compared with the Tukey test.

Performance parameters did not differ between treatment groups (data not shown). The results showed that, compared with animals fed the non-supplemented diet, phytase supplementation with Quantum® Blue at 250 FTU/kg or higher significantly increased faecal phosphorus digestibility (27, 48, 56, 59, 61 % in 0, 250, 500, 750 and 1 000 FTU/kg group, respectively) and calcium digestibility (52, 62, 65, 67, 71 % in the 0, 250, 500, 750 and 1 000 FTU/kg group, respectively).

Trial 2

A total of 32 male pigs (Du × (DL × LW)) with an initial body weight of 7.7 kg were individually caged.[65] A basal diet based on maize and soya bean meal with a total phosphorus content of 0.49 % was supplemented with Quantum® Blue 40 P to provide 0, 250, 500 or 1 000 FTU/kg feed (confirmed by analysis). Diets contained titanium dioxide as an external marker and were offered in coarse meal form. The piglets received the dietary treatments for 30 days and total faeces were collected for five days. Piglets were killed at the end of the study and ileal samples were collected. Digesta and faecal samples were used to determine organic matter, phosphorus and calcium digestibility. ANOVA was performed with the data and means were compared with the LSD test.

Performance parameters were not different between the treatments (data not shown). Results showed that the addition of Quantum® Blue phytase from 250 FTU/kg significantly increased, as compared to the non-supplemented diet, ileal phosphorus digestibility (57, 70, 73, 73 % for 0, 250, 500, or 1 000 FTU/kg, respectively) and faecal phosphorus digestibility (42, 52, 56, 60 % for 0, 250, 500, or 1 000 FTU/kg, respectively). Calcium digestibility was not different between the treatments.

Trial 3

A combined performance, digestibility and bone mineralisation study was conducted.[66] Twenty-eight-day-old piglets ((Du × LD) × (Yorkshire × LD) and (LD × (Yorkshire × LD))) were distributed in groups of two into pens. Sixteen pens per treatment were used for the performance study and eight pens per treatment for the faecal digestibility measurements. Starter and weaner diets (negative control; nc), based on barley, wheat and oats with a total phosphorus content of 0.50 % (starter) or 0.52 % (weaner) were supplemented with phytase to provide 0, 500 or 1 000 FTU/kg feed (nc0, nc500 and nc1 000 FTU/kg). Two more basal diets (starter and weaner) with a higher total phosphorus content (0.67 %) (positive control; pc) were supplemented at 0, 500 or 1 000 FTU/kg (pc0, pc500 and pc1 000). Enzyme activities were confirmed by analysis. Diets were offered for 46 days *ad libitum* in pelleted form and contained titanium dioxide as an external marker. Performance was measured throughout the study. The apparent faecal digestibility of weaner diets was determined by collecting faecal spot samples on five consecutive days (days 39–43 day of the trial). Samples were collected per pen, not individually. At the end of the experiment, 12 piglets from each of the nc0, nc500, nc1 000 and pc0 treatment groups were killed. Digesta samples from the ileum were collected and metacarpal bones III and IV from the left front foot and metatarsal bones III and IV from left hind foot were collected. Digesta and faecal samples were analysed for dry matter, organic matter, ash, phosphorus and calcium. In the ileal samples, phytate digestibility was also measured. Bones were subject to

[65] Technical dossier/Supplementary information March 2013/Annexes 51 to 55.
[66] Technical dossier/Supplementary information July 2013/Annex 5.

strength determinations and ash, phosphorus and calcium analysis. ANOVA was performed with the data and the differences between treatments were determined with the Tukey test.

No differences in the performance of the pigs were found (data not shown). Faecal digestibility of phosphorus was higher in the 500 FTU/kg group than in non-supplemented group, regardless of the phosphorus content of the basal diet (49, 61 and 68 % in the nc0, nc500 and nc1 000 FTU/kg group, respectively, and 52, 62 and 61 % in the pc0, pc500 and pc1 000 FTU/kg group, respectively). Ileal digestibility of phosphorus (45, 60, 60 and 52 % in the nc0, nc500, nc1 000 and pc0 group, respectively), bone ash (33, 35, 37, 36 % in the nc0, nc500, nc1 000 and pc0 group, respectively) and phosphorus in bones (5.6, 6.0, 6.3 and 6.1 % in the nc0, nc500, nc1 000 and pc0 group, respectively) were significantly higher in the nc500 and nc1 000 groups than in the nc0 group. Digestibility of calcium was not affected by phytase addition.

The results of the three trials showed that the supplementation of the feed with Quantum® Blue phytase at the minimum recommended dose of 250 FTU/kg resulted in a significant increase in phosphorus digestibility in two trials (trial 1 and 2). A dose of 500 FTU/kg diet resulted in a significant increase in phosphorus digestibility and bone mineralisation in another trial. Therefore, the additive has the potential to be efficacious in weaned piglets at the dose of 500 FTU/kg feed.

4.4. Efficacy for pigs for fattening

The applicant provided two short-term trials and two long-term trials.

Short-term trials

In the first short-term trial, a total of 12 castrated male growing pigs (Pi × (DE × DL)) were kept in pens until they reached 60 kg body weight (during which time they were fed a commercial feed) and then allocated to one of three dietary treatments.[67] A basal diet based on maize and soya bean meal with a total phosphorus content of 0.30 % was supplemented with Quantum® Blue 40 P to provide 0, 250 or 500 FTU/kg feed (confirmed by analysis). The diets were offered in pelleted form. The trial was run in two separate collection periods. The dietary treatment received by the pigs was changed between periods, resulting in eight replicates per treatment. Before the start of the collection periods, the animals had a nine-day period of adaptation to the diets. Two days before the collection started, the pigs were moved to cages. Total faeces were collected for five days. Feed allowance was 1 800 g/day in the first collection period and 2 000 g/day in the second. Feed refusals were quantified. Faeces were used to determine organic matter, phosphorus and calcium. ANOVA was performed with the data and means were compared with the Tukey test.

Performance parameters did not differ between treatment groups (data not shown). The results showed that, compared with the non-supplemented diet, the addition of Quantum® Blue phytase from 250 FTU/kg increased phosphorus digestibility (30, 45 and 47 % in the 0, 250 and 500 FTU/kg group, respectively). Calcium digestibility was not affected by treatments (53, 58 and 53 % in the 0, 250 and 500 FTU/kg group, respectively).

In the second short-term trial provided, castrated male pigs (Du × (DL × LW)) with an initial body weight of 28 kg were used.[68] A basal diet based on maize and soya bean meal with a total phosphorus content of 0.37 % was supplemented with Quantum® Blue 40 P to provide 0, 250, 500 or 1 000 FTU/kg feed (confirmed by analysis). The diets were offered in coarse meal form at 2.6 times the pigs' maintenance energy requirement. The trial was run in two separate collection periods. the dietary treatment received by the pigs was changed between periods, resulting in at least eight replicates per treatment (8–10). Before the start of the collection periods, a nine-day adaptation to the diets was followed. Total faeces were collected for five days. Feed refusals were quantified. Faeces

[67] Technical dossier/Section IV/Annexes IV.31 to IV.44 and supplementary information March 2013/Annex 57.
[68] Technical dossier/Supplementary information March 2013/Annex 58 to 62.

were used to determine dry matter, phosphorus and calcium. ANOVA was performed on the data and means were compared with a LSD test.

Performance parameters did not differ between treatment groups (data not shown). The results showed that, compared with the non-supplemented diet, the addition of phytase at 250 FTU/kg or above significantly increased phosphorus digestibility (34, 44, 49, and 52 % in the 0, 250, 500 and 1 000 FTU/kg group, respectively) and calcium digestibility (54, 61, 64, and 63 % in the 0, 250, 500 and 1 000 FTU/kg group, respectively).

Long-term trials

The first long-term trial was a combined performance, digestibility and bone mineralisation study that was performed with 48 pigs ((Du × LD) × (Yorkshire × LD)) with an initial body weight of 38 kg (77 days of life).[69] The animals were allocated to one of four dietary treatments (six castrated males and six females per treatment). Grower and finisher diets, based on barley, wheat, oats and soya bean meal with a total phosphorus content of 0.45 % (grower) or 0.40 % (finisher) were supplemented with Quantum® Blue 40 P to provide 0, 250 or 500 FTU/kg feed (confirmed by analysis). A positive control was also included (phosphorus content 0.51 and 0.55 % in the grower and finisher diets, respectively). Diets were offered as pellets for a total of 68 days. The performance of the pigs was measured throughout the study. Pigs were weighed at two-week intervals and at the end of the experiment. Feed consumption was recorded daily (access to feed was not *ad libitum*). During the fourth week of the study, the diets contained titanium oxide as an external marker. On five consecutive days, spot samples were collected from five gilts and five barrows in each treatment group. Faecal samples were analysed for the main dietary fractions, phosphorus and calcium. One week after the pigs had reached at least 100 kg, they were slaughtered, carcass characteristics were recorded (data not shown) and the third and fourth metacarpals were collected to measure bone breaking strength and for analysis of dry matter, ash, phosphorus and calcium (pooling the two bones). ANOVA was performed on the data and differences between means were tested with the Tukey test.

The results of the performance and bone mineralisation studies are presented in Table 6. Pigs in the 250 FTU/kg feed group ate less and grew less than pigs receiving the 0 FTU/kg diet, but the feed to gain ratio did not differ between these groups.

Table 6: Effect of Quantum® Blue on the performance and bone mineralisation of pigs for fattening

Intended dose (FTU/kg)	Body weight (kg)		Daily feed intake (kg)	Body weight gain (g/day)	Feed to gain ratio	Metacarpals (% dry matter)	
	Initial	Final				Ash	P
Negative control	39	70	2.51[a]	1 177[a]	2.14	38	6.4[b]
250	39	68	2.42[b]	1 099[c]	2.21	39	6.5[b]
500	39	70	2.48[a]	1 135[abc]	2.19	40	6.8[a]
Positive control	38	68	2.51[a]	1 153[ab]	2.19	40	6.7[a]

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

The faecal digestibility of phosphorus was higher in the 500 FTU/kg feed group than in the 0 FTU/kg group (47, 46, 54 and 44 % in the 0, 250, 500 FTU/kg feed and positive control group, respectively). Calcium digestibility was higher in the 0 and 500 FTU/kg feed groups than in the other two groups (56, 46, 54 and 46 % in the 0, 25, 500 FTU/kg feed and positive control group, respectively). Analysis of bone strength showed that bending moment in the fourth metacarpal bone was significantly lower in the 500 FTU/kg feed group than in the 0 FTU/kg group; however, bending moment in the third metacarpal bone did not differ between the groups. When considering the mean bone strength in the

[69] Technical dossier/Section IV/Annexes IV.34 to IV.37 and supplementary information March 2013/Annex 56.

two bones, no differences were found between supplemented and 0 FTU/kg groups. The 500 FTU/kg group showed a higher bone phosphorus content than the negative control.

The second long-term trial was a combined performance, digestibility and bone mineralisation trial performed with 96 castrated males (Pietrain × (Euroc × LD; 25 kg bw).[70] Pigs were allocated in groups of three to four dietary treatments (eight replicates per treatment). Starter, grower and finisher diets based on maize, wheat, barley and soya bean meal with a total phosphorus content of 0.38 % (starter), 0.37 % (grower) and 0.36 % (finisher), respectively, were supplemented with Quantum® Blue 40 P to provide 0, 250 and 500 FTU/kg (confirmed by analysis). A positive control was also included (total phosphorus content of 0.61, 0.59 and 0.48 % for starter, grower and finisher diets, respectively). Diets were offered *ad libitum* as mash and in three different periods (corresponding to 35, 65 and 115 kg body weight). Performance was measured throughout the experimental period. In the three periods, digestibility was studied by adding chromium oxide to the diets for 10 days as an external marker. Faeces, pooled per pen, were collected by rectal stimulation for three consecutive days after an adaptation period of seven days. At the end of the study, the left tibia of 10 pigs per group was collected and mineralisation was studied. ANOVA was performed with the data and means were compared by the Tukey test. The results are presented in Table 7.

Body weight gain was significantly higher and feed to gain ratio was significantly lower in pigs fed diets supplemented with Quantum® Blue phytase at 250 FTU/kg or above than in the negative control group. Phosphorus digestibility in all three periods was higher in the groups receiving phytase supplementation at 250 FTU/or above than in the 0 FTU/kg feed group (35/32/32 %, 44/39/43 %, 53/48/54 % and 40/44/43 % in the 0, 250, 500 FTU/kg and positive control group, respectively, for periods 1/2/3). The digestibility of calcium was also improved by phytase supplementation. Addition of phytase at 250 FTU/kg or above also resulted in a significant increase in bone ash and phosphorus content compared with the negative control.

Table 7: Effect of Quantum® Blue on the performance and bone mineralisation of pigs for fattening

Intended dose (FTU/kg)	Body weight (kg)		Daily feed intake (kg)	Body weight gain (g/day)	Feed to gain ratio	Bone (% dry matter)	
	Initial	Final				Ash	P
Negative control	25	112[a]	2.53	781[a]	3.25[b]	48[b]	8.0[b]
250	25	117[b]	2.54	824[b]	3.09[a]	53[a]	8.7[a]
500	25	118[bc]	2.53	833[bc]	3.03[a]	51[a]	8.7[a]
Positive control	25	120[c]	2.61	858[c]	3.05[a]	52[a]	8.7[a]

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

The results of the four trials showed that the supplementation of the feed with Quantum® Blue phytase at the minimum recommended dose of 250 FTU/kg resulted in a significant increase in faecal phosphorus digestibility in three trials (two short-term trials and in the second long-term trial). In the second short long-term trial, bone mineralisation and the performance of the pigs were improved at the same dose. Therefore, the additive has the potential to be efficacious in pigs for fattening at the dose of 250 FTU/kg feed.

4.5. Efficacy for sows

Trial 1

[70] Technical dossier/Section IV/Annexes IV.38 to IV.40.

A digestibility trial in lactating sows was performed with 24 (German Edelschwein × German Landrace) sows (seven second-parity sows and one third-parity sow per treatment) that were allocated to one of three dietary treatments.[71] A basal diet based on maize, barley and soya bean meal with a total phosphorus content of 0.34 % was supplemented with Quantum® Blue 40 P to provide 0, 250 or 500 FTU/kg feed (confirmed by analysis). Diets were offered as pellets and contained titanium dioxide as an external marker. Sows received the experimental treatment from day 1 of lactation to day 28. The maximum feed allowance was 6 000 g/day and feed was provided twice daily. Sows were weighed 20 days prior to farrowing and at the end of the experiment. Feed consumption was registered for each sow. During the last week of lactation, piglets were offered creep feed, and its consumption was recorded. Piglets were weighed on day 1 and day 27. Spot faecal samples of the sows were collected on five consecutive days from day 15 (avoiding those samples containing urine and/or feed). Faecal samples collected were subject to chemical analysis in order to calculate the digestibility of the dietary fractions and the phosphorus and calcium content. ANOVA was performed with the data and means were compared with the Tukey test.

One sow from the 500 FTU/kg group had to be withdrawn from the study because of illness. Mean sow body weight did not differ between groups either at the beginning or at the end of the study; hence, body weight loss was similar between groups. The litter size did not differ between groups at the beginning of the study (14.8, 13.1 and 12.1 piglets in the 0, 250 and 500 FTU/kg group, respectively) but at the end of lactation was higher in the non-supplemented group than in the supplemented groups (12.5 vs. 10.5). However, total litter weight gain during lactation did not differ between treatment groups (77.9, 74.6 and 81.7 kg in the 0, 250 and 500 FTU/kg group, respectively). Phosphorus digestibility was higher in the sows receiving feed supplemented with phytase than in the non-supplemented group (32.0, 44.5 and 51.0 % in the 0, 250 and 500 FTU/kg group, respectively). No effects on calcium digestibility were found (mean value 48 %). Dry matter digestibility was higher in the 500 FTU/kg group (85.5 %) as compared to the other two groups (mean value 84.5 %).

Trial 2

This trial is the tolerance trial (see section 3.2.5).[72] The dietary treatments were offered to sows from pregnancy until lactation. Farrowing and lactation performance were measured. Individual feed intake was measured during the lactation period. The digestibility of the diets was measured from day 18 to day 22 of lactation (diets contained titanium dioxide as an external marker). During this five-day period, faeces were collected after feeding; the amounts collected in the morning and afternoon were similar. ANOVA was performed with the data and means were compared with the LSD test.

The performance of the sows at farrowing or during lactation did not differ between the different treatment groups (see section 3.2.5). With the exception of the group receiving the tolerance dose, one sow per treatment group had to be culled during the study. Phosphorus digestibility was significantly higher in sows fed diets containing Quantum® Blue phytase at intended values of 250–250 000 FTU/kg (mean value 62.9 %) than in the group receiving the 0 FTU/kg feed diet (50.4 %). The mean value in the positive control group (52.7 %) was not significantly different from that in the negative control group. The same trend was found when considering the dry matter digestibility. Calcium digestibility was higher in all treatment groups than in the negative control.

Trial 3

A digestibility trial in lactating sows was performed with 47 sows (Porkuss, Danish hybrid breed; average parity 5) allocated to one of four dietary treatments. The number of sows per treatment was 10–13.[73] Sows entered the study over seven consecutive weeks and two sows per treatment were housed in the same room to avoid bias caused by room. A basal diet based on wheat, barley and soya

[71] Technical dossier/Section IV/Annexes IV.41 to IV.44 and Supplementary information March 2013/Annex 63.
[72] Technical dossier/Section IV/Annexes IV.45 to IV.48 and Supplementary information March 2013/Annex 64 and 65.
[73] Technical dossier/Section IV/Annexes IV.49 to IV.52 and Supplementary information March 2013/Annex 66.

bean meal with a total phosphorus content of 0.37–0.39 % was supplemented with Quantum® Blue 40 P to provide 0, 250 or 500 FTU/kg feed (confirmed by analysis). A diet with a total phosphorus content of 0.56 % was included as a positive control. Diets were offered as mash and contained titanium dioxide as an external marker. Sows received the experimental treatment from day 4 of lactation to day 26. The maximum feed allowance was 9 000 g/day. The lactation performance of the sows was measured. Sows were weighed seven days prior to farrowing and at the end of the experiment (backfat thickness was also measured). Feed consumption was recorded individually. During the last week of lactation, piglets were offered creep feed. Piglets were weighed on day 1 and day 26. Faecal samples from the sows were collected from day 19 of lactation and for five consecutive days. Spot fresh faecal samples were collected twice a day (avoiding those samples containing urine and/or feed). The faecal samples were subject to chemical analysis to determine phosphorus and calcium. Results were subject to ANOVA applying the Bonferroni test to compare means.

Mean sow body weight did not differ between groups either at the beginning or at the end of the study; hence, body weight loss was similar between groups. No differences in the lactation performance of the sows were found. The total litter weight at birth was 21, 23, 21, 22 kg in the 0, 250 and 500 FTU/kg and positive control group, respectively; the corresponding weights at weaning were 93, 103, 101 and 96 kg. Phosphorus digestibility was significantly higher in the groups fed diets supplemented with phytase than in the 0 FTU/kg feed group (20, 41 and 33 and 42 % in the 0, 250 and 500 FTU/kg and positive control group, respectively). Calcium digestibility was significantly higher in the 250 FTU/kg group than in the 0 and 500 FTU/kg groups but was not different to the positive control group (21, 33, 21, 39 % in the 0, 250 and 500 FTU/kg and positive control group, respectively).

Trial 4

A digestibility trial in lactating sows was performed with 43 sows (Landrace × Large White; nulliparous to parity six sows) allocated to one of four dietary treatments (the number of replicates per treatment was 9–11).[74] Sows entered the study on two separate runs. A basal diet based on maize, wheat, barley and soya bean meal with a total phosphorus content of 0.39 % was supplemented with Quantum® Blue 40 P to provide 0, 250 or 500 FTU/kg feed (confirmed by analysis). A diet with a total phosphorus content of 0.65 % was also included as a positive control. Diets were offered as mash and contained titanium dioxide as an external marker. Sows received the experimental treatment from day 2 of lactation to day 26. Feed was offered *ad libitum* and consumption was recorded individually. Sows were weighed on day 110 of gestation and at the end of the experiment (body condition score and backfat thickness were also measured). The lactation performance of the sows was measured (number of piglets per sow standardised to 12 at the beginning); the piglets were weighed on day 1 and on days 21 and 28 of lactation. Faecal samples were collected from day 21 to day 25 to determine digestibility of dietary components including phosphorus, calcium, zinc, iron and copper. Spot fresh faecal samples were collected at different times of the day. Blood samples from the sows were collected at farrowing and on day 21 of lactation; milk samples were collected on day 21 to determine zinc, iron and copper. ANOVA was performed with the data and mean values were compared using the Tukey test.

Mean sow body weight did not differ between groups either at the beginning or at the end of the study; hence, body weight loss was similar between the groups. Body condition score and backfat thickness did not differ between treatment groups. No differences in the lactation performance of the sows were detected. The average daily weight gain of piglets did not differ between the experimental groups (209, 242, 223 and 226 g/day in the 0, 250, 500 FTU/kg and positive control group, respectively), and the final number of piglets per litter did not differ (mean value 11). Phosphorus digestibility was significantly higher in the 500 FTU/kg group than in the non-supplemented group (37, 45, 46 and

[74] Technical dossier/ Supplementary information March 2013/Annex 67 to 71.

38 % in the 0, 250 and 500 FTU/kg and positive control group, respectively). No difference in the digestibility of calcium or dry matter was found.

Three studies found that phosphorus digestibility was increased by phytase supplementation from the dose of 250 FTU/kg (trials 1, 2 and 3) and one study reported improved phosphorus digestibility at 500 FTU/kg (trial 4). Furthermore, sow performance was not affected by enzyme addition in any of the trials.

4.6. Conclusions of the efficacy for the target species

Based on the results of the efficacy trials provided in chickens and turkeys for fattening, in which the additive significantly improved phosphorus utilisation, digestibility and bone mineralisation or the performance of the birds, the FEEDAP Panel concludes that the additive has the potential to be efficacious at the minimum recommended dose (250 FTU/kg feed). These conclusions can be extended to chickens reared for laying and turkeys reared for breeding. Since the mode of action of phytase is well known, and can be considered to be similar in all poultry species, the conclusions drawn for chickens and turkeys for fattening can be extrapolated to minor poultry species for fattening or reared for laying/breeding.

Based on the results of the efficacy trials provided in piglets, pigs for fattening and sows, in which the additive significantly improved phosphorus digestibility, bone mineralisation or performance parameters of the pigs, the FEEDAP Panel concludes that the additive has the potential to be efficacious at 500 FTU/kg in weaned piglets and at 250 FTU/kg in pigs for fattening and sows. The FEEDAP Panel recognises that some of the studies provided to support the efficacy of the additive in piglets and pigs for fattening do not comply with the current requirements. Considering that this application was submitted shortly after the publication of this guidance document, the Panel has exceptionally considered such studies as appropriate for the assessment of the efficacy of the additive.

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[75] and by Good Manufacturing Practice.

CONCLUSIONS

The solid and the liquid formulations are considered equivalent in terms of safety and efficacy for the target species, provided that the same dose in enzyme units applies.

Neither the production organism nor recombinant DNA was detected in the final product. The final product does not raise a safety concern with regard to the genetic modification.

Tolerance trials in chickens and turkeys for fattening, weaned piglets and sows were provided. The results showed that the animals tolerated well 100-fold the maximum recommended dosage (2 500 FTU/kg for poultry and 1 750 FTU/kg for pigs). Therefore, the FEEDAP Panel concludes that the additive is safe for those target species at the corresponding maximum recommended dose. The conclusions reached in chickens and turkeys for fattening can be extended to chickens reared for laying and turkeys reared for breeding, provided that the same dose applies. Similarly, the conclusions reached in piglets can be extended to pigs for fattening, provided that the same dose applies. Considering the margin of safety demonstrated in major poultry species, the conclusions on the safety for those species can be extrapolated to minor poultry species for fattening or reared for laying/breeding.

[75] 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.

The fermentation product that is used to prepare the final formulations of Quantum® Blue resulted negative in genotoxicity tests. The results obtained in a subchronic oral toxicity study raised no concerns regarding the product. Therefore, based on the toxicological studies performed with the fermentation product, the FEEDAP Panel concludes that the additive is of no concern regarding consumer safety.

Three final formulations, Quantum® Blue 5 G, 40 P and 10 L, were tested for skin and eye irritation and skin sensitisation. The results showed no evidence of dermal or eye irritation or skin sensitisation. The other final liquid formulation, Quantum® Blue 5 L, was not tested, but based on its composition its irritant potential or skin sensitisation potential is unlikely to be significantly different from that of its tested counterpart (Quantum® Blue 10 L). Taking account of the proteinaceous nature of its active substance, the additive is to be considered a potential respiratory sensitiser.

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

Based on the results of the efficacy trials carried out in chickens and turkeys for fattening, in which the additive significantly improved phosphorus utilisation, digestibility and bone mineralisation or the performance of the birds, the FEEDAP Panel concludes that the additive has the potential to be efficacious at the minimum recommended dose (250 FTU/kg feed). These conclusions can be extended to chickens reared for laying and turkeys reared for breeding. Since the mode of action of the phytase is well known and can be considered to be similar in all poultry species, the conclusions drawn for chickens and turkeys for fattening can be extrapolated to minor poultry species for fattening or reared for laying/breeding. Based on the results of the efficacy trials provided in piglets, pigs for fattening and sows, in which the additive significantly improved phosphorus digestibility, phosphorus retention or performance parameters, the FEEDAP Panel concludes that the additive has the potential to be efficacious at 500 FTU/kg in weaned piglets and at 250 FTU/kg in pigs for fattening and sows.

The use of the additive allows the use of diets with a lower level of inorganic phosphorus, which may in turn reduce the excretion of phosphorus.

DOCUMENTATION PROVIDED TO EFSA

1. Quantum® Blue for poultry and pigs. April 2012. Submitted by Roal Oy.
2. Quantum® Blue for poultry and pigs. Supplementary information. March 2013. Submitted by Roal Oy.
3. Quantum® Blue for poultry and pigs. Supplementary information. June 2013. Submitted by Roal Oy.
4. Quantum® Blue for poultry and pigs. Supplementary information. July 2013. Submitted by Roal Oy.
5. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Quantum® Blue.
6. Comments from Member States received through the ScienceNet.

REFERENCES

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2012. Guidance for the preparation of dossier for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. doi:10.2903/j.efsa.2012.2536

APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Quantum® Blue[76]

In the current two applications (FAD-2012-0015 and FAD-2012-0033) authorisation is sought under article 4(1) for *Quantum Blue 5 L, 10 L, 5 G* and *40 P* under the category/functional group 4(c) "zootechnical additives"/"substances which favourable affect the environment", according to the classification system of Annex I of Regulation (EC) No 1831/2003.

According to the Applicant, the active agent of all *Quantum Blue* products is *6-phytase* (EC 3.1.3.26) produced by the strain *Trichoderma reesei* (CBS 126897). The authorisation is sought for the use of the *feed additive* for a variety of animal species. According to the Applicant, *Quantum Blue 5 L* and *10 L* are liquid preparations with a guaranteed minimum enzyme activity of 5000 and 10000 FTU/g, respectively, while *Quantum Blue 5 G* and *40 P* are a granulate (G) or powder (P) preparation with a guaranteed minimum enzyme activity of 5000 and 40000 FTU/g, respectively. *Quantum Blue* preparations are intended to be used in *premixtures* and/or complete *feedingstuffs* to obtain minimum enzyme activities for 6-phytase of 150 and 250 FTU/kg *feedingstuffs* depending on the target species. The dry products can be mixed with the final feed or with the mineral *premixtures*, while the liquid product is to be sprayed onto feed pellets.

The Applicant used the enzyme activity units as defined in the EN ISO 30024:

- One *6-phytase* unit (FTU) is the amount of enzyme which liberates 1 micromole of inorganic phosphate from sodium phytate in one minute at 37°C and pH 5.5.

For the determination of the activity of *6-phytase* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant proposed an in-house developed and validated colorimetric method measuring the inorganic phosphate released by the enzyme from the sodium phytate substrate. On the request of the EURL the Applicant applied the internationally recognised ring-trial validated colorimetric CEN method (EN ISO 30024) for the determination of *6-phytase* in the *feed additive*, *premixtures* and *feedingstuffs* samples containing *Quantum Blue* products and provided performance characteristics similar to those reported in the EN ISO 30024 standard:

- a precision (repeatability and reproducibility) ranging from 1.2 to 5.5 %;

- a *recovery* rate ranging from 80.4 to 102.4 %; and - a limit of quantification of 101 FTU/kg *feedingstuffs*.

Based on the experimental evidence and the performance characteristics presented, the EURL recommends for official control the EN ISO 30024 method, for the determination of the activity of the *6-phytase* in the *feed additive*, *premixtures* and *feedingstuffs*.

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

[76] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2012-0015-Quantum%20Blue.doc[1].pdf