

SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of Quantum[®] Blue (6-phytase) as a feed additive for laying hens and minor laying poultry species¹

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)^{2,3}

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ABSTRACT

Quantum[®] Blue is a 6-phytase preparation that is available in solid and liquid forms. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of this product as a feed additive for poultry (except laying hens) and pigs, including the assessment of the safety for the consumer, the user and the environment, as well as the safety aspects of the genetic modification. The FEEDAP Panel considers, therefore, that the safety aspects, other than for the new target species, are covered in the previous opinion and would not be affected by the requested extension of use. The present assessment focuses on the safety and efficacy of this enzyme preparation for the new target species. The results from a tolerance trial in laying hens showed that the hens tolerated well 100-fold the maximum recommended dose; therefore, the FEEDAP Panel concludes that the additive is safe for laying hens at the maximum recommended dose (2 500 FTU/kg feed). Given the wide margin of safety shown in laying hens, the FEEDAP Panel considers that the additive is safe for minor laying poultry species, provided that the same dose applies. The results from three trials showed that the addition of Quantum[®] Blue phytase resulted in an increase in bone mineralisation, ileal digestibility, phosphorus utilisation and performance of the laying hens. The FEEDAP Panel concludes that the additive has the potential to be efficacious in laying hens at the dose of 150 FTU/kg feed. The FEEDAP Panel considers that the conclusions drawn for laying hens can be extrapolated to minor laying poultry species at the same dose.

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KEY WORDS

zootechnical additive, substances which favourably affect the environment, safety, efficacy, laying hens

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Quantum[®] Blue (6-phytase) as a feed additive for laying hens and minor laying poultry species.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of this additive as a feed additive for poultry (except laying hens) and pigs, including the assessment of the safety for the consumer, the user and the environment, as well as the safety aspects of the genetic modification. The FEEDAP Panel considers that the safety aspects, other than for the new target species, are covered in the previous opinion and would not be affected by the requested extension of use. Therefore, the present opinion focuses on the safety and efficacy of this enzyme preparation for the new target species.

The solid and liquid formulations of the additive are considered equivalent in terms of safety and efficacy for the target species.

The results from a tolerance trial in laying hens showed that the hens tolerated well 100-fold the maximum recommended dose; therefore, the FEEDAP Panel concludes that the additive is safe for laying hens at the maximum recommended dose (2 500 phytase units (FTU)/kg feed). Given the wide margin of safety shown in laying hens, the FEEDAP Panel considers that the additive is safe for minor laying poultry species, provided that the same dose applies.

The results from three trials showed that the addition of Quantum[®] Blue phytase resulted in an increase in bone mineralisation, ileal digestibility, phosphorus utilisation and performance of the laying hens. The FEEDAP Panel concludes that the additive has the potential to be efficacious in laying hens at the dose of 150 FTU/kg feed. The FEEDAP Panel considers that the conclusions drawn for laying hens can be extrapolated to minor laying poultry species at the same dose.

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BACKGROUND

Regulation (EC) No 1831/2003⁴ 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 Roal Oy⁵ for authorisation of the product Quantum[®] Blue, 6-phytase, when used as a feed additive for laying hens and minor laying poultry species (category: zootechnical additives; 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.⁶ According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 23 January 2013.

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. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) adopted an opinion on Quantum[®] Blue, including the evaluation of the safety for the consumers, users and the environment and the safety of the genetic modification (EFSA FEEDAP Panel, 2013).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and the efficacy Quantum[®] Blue, 6-phytase, when used under the conditions described in Table 1.

⁴ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

⁵ Roal Oy, Tykkimäentie 15, 05200 Rajamäki, Finland.

⁶ EFSA Dossier reference: FAD-2012-0033.

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 <i>Trichoderma reesei</i> (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 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		
Laying hens	-	150 FTU	-	-
Minor laying poultry species	-	150 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: Laying hens: 150 – 2 500 FTU/kg Minor laying poultry species: 150 – 2 500 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 presented in liquid (Quantum[®] Blue 5 L and Quantum[®] Blue 10 L) and solid forms (Quantum[®] Blue 5 G and Quantum[®] Blue 40 P). 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 laying hens and minor laying poultry species.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of this product for use in poultry (except laying hens) and pigs, including the assessment of the safety for the consumer, the user and the environment, as well as the safety aspects of the genetic modification (EFSA FEEDAP Panel, 2013). The FEEDAP Panel considers that the safety aspects, other than for the new target species, are covered in the previous opinion and would not be affected by the requested extension of use. Therefore, the present opinion focuses on the safety and efficacy of this enzyme preparation for the new target species.

2. Conditions of use

The additive is to be used in feed for laying hens or for minor laying poultry species at a dose range of 150 to 2 500 phytase units (FTU)/kg feed.

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

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

4. Safety for the target species

4.1. Safety for laying hens

A total of 720 21-week-old Hy-Line brown hens were distributed into 144 cages of five hens and allocated to four dietary treatments.⁷ One replicate consisted of three cages, representing a total of 12 replicates per treatment. A basal diet based on maize and soya bean meal with a total phosphorus content of 3.3 g/kg was supplemented with phytase concentrate to provide 0 (negative control), 2 500 (1×) or 250 000 (100×) FTU/kg feed. A positive control was also considered (5.4 g total P/kg feed). Phytase activity in feed was < 50, 2 400, 243 000 and < 50 FTU/kg feed for negative control, 1×, 100× and positive control, respectively. Feed was offered in mash form for 56 days. Health, feed intake and laying performance were monitored throughout the study. Egg quality parameters (number of broken, dirty or faulty eggs, egg yolk colour, albumen height and shell strength) were also measured. An analysis of variance (ANOVA) was performed with the data.

Mortality was low (two, one, one and zero hens in the negative control, 1×, 100× and positive control groups, respectively). Feed intake (104, 106, 106 and 108 g/day in the negative control, 1×, 100× and positive control groups, respectively), egg mass output (54, 56, 56 and 56 g/day, respectively) and feed to egg ratio (1.91, 1.90, 1.91 and 1.91, respectively) were not modified by the addition of phytase compared with the negative control diet. Laying rate was improved by phytase supplementation (90, 94, 93 and 93 % in the negative control, 1×, 100× and positive control groups, respectively). Weight gain of the hens was higher in the phytase groups than in the negative control group (~ 105 vs. 28 g). Egg shell strength was higher in the 100× group than in the other groups (~ 4.9 vs. 4.5 kg/cm²).

⁷ Technical dossier/Section III/Annexes 1 to 4 and supplementary information April 2013/Annexes 1 and 2.

The hens tolerated well 100-fold the maximum recommended dose; therefore, the FEEDAP Panel concludes that the additive is safe for laying hens at the maximum recommended dose.

4.2. Safety for minor laying poultry species

Given the wide margin of safety shown in the tolerance trial provided in laying hens, in which the animals tolerated well 100-fold the maximum recommended dose, the FEEDAP Panel considers that the additive is safe for minor laying poultry species, provided that the same dose applies.

5. Efficacy

5.1. Efficacy for laying hens

The applicant provided three efficacy trials.

Trial 1

A total of 72 35-week-old Lohmann Brown hens were distributed individually in cages and allocated to six dietary treatments (12 replicates per treatment).⁸ A basal diet based on maize, wheat, barley and soya bean meal with a total phosphorus content of 2.5 g/kg feed was supplemented with Quantum[®] Blue 40 P to provide 0 (negative control), 150, 300, 600 or 1 200 FTU/kg feed. A positive control was also included (5.3 g total P/kg feed). Phytase activity in feed was 68, 196, 295, 616, 1 110 and < 50 FTU/kg feed in the negative control, 150, 300, 600, 1 200 FTU/kg feed and positive control groups, respectively. Feed was offered in mash form for 42 days and chromic oxide was used as a marker. Health, feed intake, laying performance and egg shell stability were monitored throughout the study. At the end of the study, all the hens were killed and digesta samples from the ileum and the tibia were collected to determine ileal digestibility and bone mineralisation. An ANOVA was performed with the results and differences between the group means were studied with a Tukey test.

No hens died during the study. The performance of the hens was up to the standards and no differences between the treatments were found. Dietary supplementation with phytase significantly improved the ileal digestibility of phosphorus from the addition of 150 FTU/kg feed compared with the negative control (33, 44, 51, 57, 56 and 54 % in the 0, 150, 300, 600, 1 200 FTU/kg feed and positive control groups, respectively). Tibia ash (437, 445, 457, 474, 470 and 470 g/kg defatted dry bone weight) and phosphorus content (70, 73, 74, 78, 78 and 77 g/kg defatted dry bone weight) were significantly higher in the groups receiving 300 FTU/kg feed or more compared with the negative control.

Trial 2

A total of 168 40-week-old Lohmann Brown hens were distributed individually in cages and allocated to seven dietary treatments (24 replicates per treatment).⁹ A basal diet based on maize and soya bean meal with a total phosphorus content of 2.94 g/kg feed was supplemented with Quantum[®] Blue 40 P to provide 0 (negative control), 150, 300, 600, 900 or 1 200 FTU/kg feed. A positive control was also included (4.7 g total P/kg feed). Phytase activity in feed was < 50, 196, 314, 654, 950, 1 340 and < 50 FTU/kg feed in the negative control, 150, 300, 600, 900, 1 200 FTU/kg feed and positive control groups, respectively. Feed was offered in mash form for 28 days; titanium dioxide was used as a marker. Feed intake and laying performance were recorded throughout the study. Over the last five days of the feeding period, excreta of 11 laying hens per treatment were collected in order to evaluate phosphorus utilisation. At the end of the experimental period, all hens were killed and ileal contents were collected to evaluate ileal digestibility; samples from two hens were pooled (12 samples per treatment). An ANOVA was performed with the results and differences between the group means were studied with a Tukey test.

⁸ Technical dossier/Section IV/Annexes 1 to 3 and supplementary information April 2013/Annex 3.

⁹ Technical dossier/Section IV/Annexes 4 to 6 and supplementary information April 2013/Annex 4.

No hens died during the experiment. Laying performance of the hens was up to the standards and not different between treatments with the exception of mean egg weight, which was higher in the 600 FTU/kg group than in the negative control (63.6 g vs. 60.3 g). Ileal digestibility and utilisation (excreta) of phosphorus were higher in the groups receiving supplementation at 150 FTU/kg or more compared with the negative control group (35/30, 43/39, 46/43, 44/43, 47/43, 47/45 and 48/45 % ileal digestibility/utilisation for the 0, 150, 300, 600, 900, 1 200 FTU/kg feed and positive control groups, respectively).

Trial 3

This trial was a combined performance and balance study. A total of 180 48-week-old Hy-Line Brown hens were caged in groups of three hens in 60 cages (12 replicates per treatment).¹⁰ A basal diet based on maize and soya bean meal with a total phosphorus content of 3.2 g/kg feed was supplemented with Quantum® Blue 40 P to provide 0 (negative control), 150, 300 or 1 200 FTU/kg feed. A positive control diet was also included (4.9 g total P/kg feed). Phytase activity in feed was < 50, 134, 203, 909 and < 50 FTU/kg feed in the negative control, 150, 300, 1 200 FTU/kg feed and positive control groups, respectively. Feed was offered as mash for 26 weeks. Laying performance was measured throughout the study, as were egg shell thickness and breakability. Out of the 12 cages per treatment, 10 were selected for the balance study starting on week 44 of laying (equivalent to week 64 of age). The hens were in an adaptation period for four days and thereafter a seven-day collection period of excreta followed. The diets used were the same as the ones used in the performance study, but contained titanium dioxide in order to study the utilisation of phosphorus. An ANOVA was performed with the results and differences from the control were determined with a Dunnett test. The results are shown in Table 2.

Laying rate, egg mass and phosphorus utilisation were higher in the groups receiving the phytase at a dose of 150 FTU/kg feed or more compared with the negative control.

Table 2: Effect of Quantum® Blue on the performance of laying hens, egg shell parameters and phosphorus utilisation

Intended dose (FTU/kg)	Laying rate (%)	Egg mass (g/hen)	Feed intake (g/day)	Feed- to-egg mass ratio	Egg shell		Phosphorus utilisation (%)
					Thickness (mm)	Breaking strength (kp)	
Negative control	75	8 476	103	2.23	0.34	3.31	33
150	81*	9 148*	108	2.16	0.34	3.11	40*
300	82*	9 215*	106	2.09	0.34	3.25	41*
1 200	84*	9 393*	107	2.08	0.34	3.22	39*
Positive control	82*	9 103*	106	2.12	0.33	3.27	33

*Values within one column are significantly different compared with the negative control ($P < 0.05$).

The results showed that the addition of Quantum® Blue phytase led to an increase in ileal digestibility (trials 1 and 2), phosphorus utilisation (trials 2 and 3) and performance of the laying hens (trial 3) at the dose of 150 FTU/kg feed, and in bone mineralisation at the dose of 300 FTU/kg feed (trial 1). Therefore, the FEEDAP Panel concludes that the additive has the potential to be efficacious in laying hens at the dose of 150 FTU/kg feed.

¹⁰ Technical dossier/Section IV/Annexes 7 to 10 and supplementary information April 2013/Annexes 5 to 6.

5.2. Efficacy for minor laying poultry species

No studies carried out in minor laying poultry species have been presented. The mode of action of phytase is well known and can be considered to be similar in all poultry species; therefore, the FEEDAP Panel considers that the conclusions drawn for laying hens can be extrapolated to minor laying poultry species at the same dose.

6. 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 Regulation¹¹ and Good Manufacturing Practice.

CONCLUSIONS

The solid and liquid formulations of the additive are considered equivalent in terms of safety and efficacy for the target species.

Based on the results from a tolerance trial, in which laying hens showed that the hens tolerated well 100-fold the maximum recommended dose, the FEEDAP Panel concludes that the additive is safe for laying hens at the maximum recommended dose (2 500 FTU/kg feed). Given the wide margin of safety shown in laying hens, the FEEDAP Panel considers that the additive is safe for minor laying poultry species, provided that the same dose applies.

Results from three trials showed that the addition of Quantum[®] Blue phytase resulted in an increase in bone mineralisation, ileal digestibility, phosphorus utilisation and performance of the laying hens. The FEEDAP Panel concludes that the additive has the potential to be efficacious in laying hens at the dose of 150 FTU/kg feed. The FEEDAP Panel considers that the conclusions drawn for laying hens can be extrapolated to minor laying poultry species at the same dose.

DOCUMENTATION PROVIDED TO EFSA

1. Quantum[®] Blue for laying hens and minor laying poultry species. September 2012. Submitted by Roal Oy.
2. Quantum[®] Blue for laying hens and minor laying poultry species. Supplementary information. April 2013. Submitted by Roal Oy.
3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Quantum[®] Blue.
4. Comments from Member States received through the ScienceNet.

REFERENCES

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of Quantum[®] Blue (6-phytase) as a feed additive for poultry (except laying hens) and pigs. EFSA Journal 2013;11(10):3364, 3 pp. doi:10.2903/j.efsa.2013.3364

¹¹ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Quantum® Blue¹²

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 favourably 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.

¹² 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](http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2012-0015-Quantum%20Blue.doc[1].pdf)