REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment on the Safety and Efficacy of an Additive Consisting of 6-Phytase Produced by *Trichoderma Reesei* (Quantum® Blue) as a Feed Additive for Use in Fin Fish (RP1275)

Food Standards Agency[1], Food Standards Scotland[2]

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Keywords: Regulated Products, Feed Additives, Safety Assessment

https://doi.org/10.46756/001c.137867

FSA Research and Evidence

An application was submitted to the Food Standards Agency (FSA) in September 2021 from Roal Oy ('the applicant') for the new use (extension of species) of an additive consisting of 6-phytase produced by *Trichoderma reesei* (Quantum® Blue) under the category 'zootechnical', functional group 'digestibility enhancer'. The additive is proposed to be used in feed for fin fish at a minimum recommended enzyme activity of 500 FTU/kg feed and at a recommended level ranging between 500 – 2,500 FTU/kg complete feed.

To support the FSA and Food Standards Scotland (FSS) in evaluating the dossier, the Advisory Committee on Animal Feedingstuffs (ACAF) were asked to review the dossier and the supplementary information from the applicant.

The FSA/FSS concluded, based on the ACAF's advice, that the additive was correctly identified and characterised, and that it is stable at room temperature for 12 months and in animal feed for 2 months.

The ACAF concluded that the additive is safe for the target animals, the consumers, and the environment. The additive is not irritant to skin or eyes but should be treated as a potential respiratory sensitiser. The additive is not a skin-sensitiser.

The ACAF concluded that Quantum® Blue is efficacious in salmonids at an inclusion of 2,500 FTU/kg; however, they could not conclude on efficacy in other species of fin fish at a lower inclusion of 500 FTU/kg. Regarding species of fin fish other than salmonids, the Committee concluded that the additive has the potential to be efficacious at levels of 2,500 FTU/kg of Quantum® Blue.

The views of ACAF have been taken into account in the safety assessment which represents the opinion of the FSA and FSS.

This is a joint FSA and FSS publication.

1. Introduction

The FSA and FSS have undertaken an assessment for a feed additive (Quantum® Blue) containing 6-phytase produced by *Trichoderma reesei* (Roal Oy in Tykkimäentie 15b, 05200 Rajamäki, Finland) under assimilated Regulation (EC) No 1831/2003. The application sought a new use for fin fish. The additive falls under the category 'zootechnical additive', functional group 'digestibility enhancers'.

To support the safety assessment by the FSA and FSS, the ACAF provided advice to the FSA and FSS outlined in this document.

In line with Article 8 of 1831/2003, the assessment has considered and concluded the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by EFSA for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

The dossier was evaluated by the Advisory Committee on Animal Feedingstuffs (ACAF) at its September 2023 meeting, after which a request for further information was communicated to the applicant. The applicant's response to this request and subsequent requests were evaluated by ACAF at its January and December 2024 meetings.

The views of ACAF have been taken into account in this safety assessment which represents the opinion of the FSA/FSS.

Table 1. Table showing products included in this assessment.

Title	Product type	Intended use/s	Intended dose/intake
Quantum® Blue 5 L, 10 L, 5 G and 40 P	Feed additive	Zootechnical additive	500 – 2,500 FTU/kg complete feed

2. Assessment

2.1. Section II: Identity, characterisation and conditions of use

Quantum® Blue contains 6-phytase produced by fermentation with a genetically modified strain of *Trichoderma reesei* and is presented in four different forms: two liquid forms (Quantum® Blue 5 L and Quantum® Blue 10 L), a concentrated powder (Quantum® Blue 40 P) and a dry granulate (Quantum® Blue 5 G). The applicant provided data from three batches supporting the identity of each form of the additive (Table 2 to Table 5).

Table 2. Identity table for Quantum® Blue 5 L.

Composition	
Phytase activity (FTU/g)	Min. 5,000
TOS	21.4 – 40.4
Water/moisture (%)	59.6 - 78.6
Appearance	
Brown aqueous liquid	
Chemical-physical properties	
Density (kg/L)	1.12
Viscosity (mPas)	3.07
Surface tension (mN/m)	42.2
Impurities	
Arsenic	< 0.5 mg/kg
Lead	< 0.05 mg/kg
Cadmium	< 0.03 mg/kg
Mercury	< 0.05 mg/kg
E. coli	Absent in 25 g
Salmonella spp.	Absent in 25 g
Coliforms	< 30 CFU/g
Yeast and moulds	< 1,000 CFU/g
Aflatoxin B1	< 0.05 µg/kg
Aflatoxin B2	< 0.05 µg/kg
Aflatoxin G1	< 0.05 µg/kg
Aflatoxin G2	< 0.05 µg/kg
Sterigmatocystin	< 10 µg/kg
Ochratoxin A	< 0.5 µg/kg
Deoxynivalenol	< 40 µg/kg
T2-Toxin	< 10 µg/kg
Zearalenone	< 10 µg/kg
Fumonisin B2	< 10 µg/kg

FTU = Phytase activity; CFU = colony forming units; TOS = total organic solids.

Table 3. Identity table for Quantum® Blue 10 L.

Composition	
Phytase activity (FTU/g)	Min. 10,000
TOS	21.4 – 40.4
Water/moisture (%)	59.6 – 78.6
Appearance	
Brown aqueous liquid	
Chemical-physical properties	
Density (kg/L)	1.13
Viscosity (mPas)	3.27
Surface tension (mN/m)	41.3
Impurities	
Arsenic	< 0.5 mg/kg
Lead	< 0.05 mg/kg
Cadmium	< 0.03 mg/kg
Mercury	< 0.05 mg/kg
E. coli	Absent in 25 g
Salmonella spp.	Absent in 25 g
Coliforms	< 30 CFU/g
Yeast and moulds	< 1,000 CFU/g
Aflatoxin B1	< 0.05 µg/kg
Aflatoxin B2	< 0.05 µg/kg
Aflatoxin G1	< 0.05 µg/kg
Aflatoxin G2	< 0.05 µg/kg
Sterigmatocystin	< 10 µg/kg
Ochratoxin A	< 2 µg/kg
Deoxynivalenol	<40 – 301 µg/kg
T2-Toxin	< 20 µg/kg
Zearalenone	< 50 µg/kg
Fumonisin B2	< 10 µg/kg

FTU = Phytase activity ; CFU = colony forming units; TOS = total organic solids.

The Committee requested more recent data relating to the qualitative and quantitative compositions of the products, including impurity testing. More recent studies related to the following sections were also requested: absence of antibiotic activity, absence of the production strain, absence of mycotoxins and absence of deoxyribonucleic acid (DNA) from the production strain. Upon receiving this information, members were satisfied that the additive had been fully identified and characterised.

Only one batch of each of the solid forms of the additive was used to determine dusting potential. Three batches of each were subsequently provided by the applicant, allowing the Committee to conclude that neither Quantum® Blue 5 G nor Quantum® Blue 40 P were expected to form a dust during normal handling.

Table 4. Identity table for Quantum® Blue 5 G.

Composition	
Phytase activity (FTU/g)	Min. 5,000
TOS	93
Water/moisture (%)	7
Appearance	
Light brown granulate	
Chemical-physical properties	
Dusting potential	0.00 g/m^3
Particle size distribution	> 250 µm – 97.34 % < 63 µm – 0.00 % < 1µm – 0.00 %
Bulk density	0.7 kg/L
Impurities	
Arsenic	< 0.5 mg/kg
Lead	< 0.05 mg/kg
Cadmium	0.06 – 0.08 mg/kg
Mercury	< 0.05 mg/kg
E. coli	Absent in 25 g
Salmonella spp.	Absent in 25 g
Coliforms	< 30 CFU/g
Yeast and moulds	< 1,000 CFU/g
Aflatoxin B1	< 0.05 µg/kg
Aflatoxin B2	< 0.05 µg/kg
Aflatoxin G1	< 0.05 µg/kg
Aflatoxin G2	< 0.05 µg/kg
Sterigmatocystin	< 10 µg/kg
Ochratoxin A	< 2 µg/kg
Deoxynivalenol	55 – 274 µg/kg
T2-Toxin	< 20 µg/kg
Zearalenone	< 50 µg/kg
Fumonisin B2	< 10 µg/kg

FTU = Phytase activity ; CFU = colony forming units; TSO = total organic solids.

Queries were initially raised regarding the shelf-life of the additive; however further data was provided and the Committee concluded that the additive has a shelf-life of 12 months as activity remained above the minimum declared. Additional stability studies were provided, allowing the Committee to conclude that the additive is stable in animal feed for up to 2 months. The applicant provided results from an assay to demonstrate phytase activity of the feed additive in water.

Table 5. Identity table for Quantum® Blue 40 P.

Composition	
Phytase activity (FTU/g)	Min. 40,000
TOS	95
Water/moisture (%)	5
Appearance	
Light brown powder	
Chemical-physical properties	
Dusting potential	0.00 g/m^3
Particle size distribution	> 250 µm – 14.87 % < 63 µm – 9.65 % < 1µm – 0.00 %
Bulk density	0.8 kg/L
Impurities	
Arsenic	< 0.5 mg/kg
Lead	< 0.05 mg/kg
Cadmium	< 0.03 mg/kg
Mercury	< 0.05 mg/kg
E. coli	Absent in 25 g
Salmonella spp.	Absent in 25 g
Coliforms	< 30 CFU/g
Yeast and moulds	< 1,000 CFU/g
Aflatoxin B1	< 0.05 µg/kg
Aflatoxin B2	< 0.05 µg/kg
Aflatoxin G1	< 0.05 µg/kg
Aflatoxin G2	< 0.05 µg/kg
Sterigmatocystin	< 10 µg/kg
Ochratoxin A	< 2 µg/kg
Deoxynivalenol	59 – 93 µg/kg
T2-Toxin	< 20 µg/kg
Zearalenone	< 50 µg/kg
Fumonisin B2	< 10 µg/kg

FTU = Phytase activity; CFU = colony forming units; TOS = total organic solids.

The proposed conditions of use of the additive are described in Table 6.

Table 6. Conditions of use of Quantum® Blue proposed by the applicant.

Species or category of animal	Min. content in feed	Max. content in feed	Recommended content
Finfish	500 FTU/kg of complete feeding stuff	-	500 – 2,500 FTU/kg of complete feeding stuff
Other provisions and additional requirements for the labelling			
Specific conditions or restrictions for use	Store below 23°C. Shelf-life of 12 months.		
Specific conditions or restrictions for handling	For user safety – inhalation of enzyme dust or aerosol may cause respiratory allergy in susceptible individuals. Therefore, inhalation should be avoided by technical measures, e.g. good ventilation and respiratory protection.		

FTU = Phytase activity

As discussed further in Section 2.3, the ACAF were able to conclude positively on efficacy in salmonids when used at the lower level of 500 FTU/kg of additive, however, were unable to conclude on efficacy for other species of fin fish when used at this lower level. For these species other than fin fish, the ACAF concluded that the additive is only potentially efficacious at levels of 2,500 FTU/kg of Quantum® Blue.

2.1.1. Conclusions on Section II

The ACAF concluded that the additive was fully identified and characterised. Members concluded on a shelf-life of 12 months for the additive and concluded on stability in animal feed for 2 months. The Committee accepted the proposed conditions of use, however, were unable to conclude on efficacy for species of fin fish other than salmonids at the lower dose of 500 FTU/kg. No further concerns were raised for Section II of the dossier.

2.2. Section III: Safety

2.2.1. Safety for the target species

Tolerance studies were performed with rainbow trout and European sea bass using either a highly concentrated liquid of Quantum® Blue and/or Quantum® 5 L.

A trial was run for 90 days to assess the tolerance of rainbow trout to the inclusion of Quantum® Blue. Members requested further information relating to the diet used in this tolerance study and its formulation, including how the stability and homogeneity of the diet was maintained

throughout the trial. Upon receiving the requested information, the ACAF were able to conclude that feeding the tolerance dose of phytase did not have any negative effect on the performance of trout.

Another trial was run for 91 days to assess the tolerance of European sea bass to the inclusion of Quantum® Blue. The Committee concluded that the tolerance dose of phytase did not have a negative effect on sea bass performance.

The ACAF concluded that these tolerance studies demonstrate that the additive is well tolerated and is therefore safe for the target species under the proposed conditions of use.

2.2.2. Safety for the consumer

The applicant provided four studies to demonstrate safety for the consumer:

- Genotoxicity – Bacterial Reverse Mutation Test (OECD Guideline 471);
- Genotoxicity – *In Vitro* Chromosomal Aberration Test (OECD Guideline 473);
- Genotoxicity – Mammalian Erythrocyte Micronucleus Test (OECD Guideline 474);
- Sub-chronic oral toxicity – Repeated Dose 90-Day Oral Toxicity Study in Rodents (OECD Guideline 408).

All studies were performed using the dried phytase component of Quantum® Blue.

No evidence of mutagenicity was observed in the bacterial reverse mutation test, and no evidence of clastogenicity was seen in Chinese hamster V79 cells *in vitro*. However, members were concerned that the *in vivo* micronucleus test failed to prove that there was exposure of the target tissue to the additive; therefore the applicant provided results for an *in vitro* micronucleus test. The negative results of this micronucleus assay when viewed alongside results from other genotoxicity studies enabled the Committee to conclude that the additive is not mutagenic.

The 90-day sub-chronic toxicity study in rats observed no signs of toxicity at the maximum dose of 1,000 mg/kg bw/day. The applicant proposed a no observed effect level (NOEL) of 300 mg/kg bw/day based on bodyweight and bodyweight gain and serum concentrations of sodium and of chloride. A no observed adverse effect level (NOAEL) of 1,000 mg/kg bw/day was also proposed. The Committee accept a NOEL of 300 mg/kg bw/day and a NOAEL of 1,000 mg/kg bw/day.

The ACAF therefore conclude that all four forms of the additive of Quantum® Blue could be considered safe for the consumer.

2.2.3. Safety for the user

The applicant presented the following tests to evaluate the safety of the additive for the user/worker:

- Acute dermal irritation/corrosion study (OECD Guideline 404);
- Acute eye irritation/corrosion study (OECD Guideline 405);
- Skin sensitisation study (local lymph node assay), (OECD Guideline 429).

Three forms of the additive were used as the test substances in each of the studies: Quantum® Blue 5G, Quantum® Blue 10 L and Quantum® Blue 40 P. Regarding skin irritation, all three forms produced signs of mild irritation that were reversible; therefore they can be regarded as non-irritant to skin. For eye irritation, all test substances initially produced conjunctival redness and chemosis, which disappeared after one day; therefore they can be regarded as non-irritant to eyes. Additionally, all test substances were determined not to be skin sensitisers.

All results could be extrapolated to include Quantum Blue 5 L; therefore all four products were concluded to be non-irritant to skin and eyes, as well as not being skin sensitisers.

The applicant had not initially provided information regarding the additive's potential for inhalation toxicity. Upon receiving further information, the Committee agreed with the applicant that the potential for inhalation toxicity is negligible given the low dusting potential. However, the additive is to be considered a respiratory sensitiser due to its proteinaceous nature.

2.2.4. Safety for the environment

The applicant stated there is no expected risk to the environment, as the additive is a protein and will therefore be degraded as it passes through the digestive tract. Although phytases are generally considered low risk to the environment if used correctly, the Committee decided that a Phase 1 environment risk assessment was necessary. Upon receiving this, the ACAF concluded that the additive can be considered safe for the environment.

2.2.5. Conclusions on safety

The ACAF concluded that the additive is safe for the target animals, the consumers, and the environment.

The additive is not irritant to skin or eyes but should be treated as a potential respiratory sensitiser. The additive is not a skin-sensitiser.

2.3. Section IV: Efficacy

The applicant presented four efficacy studies in salmonids (rainbow trout) and three studies in other species: sea bream, turbot and sea bass. For most of these studies, two of the groups were supplemented with 500 or 2,500 FTU/kg of Quantum® Blue.

The Committee concluded that the additive is efficacious in salmonids at a dose of 500 FTU/kg of additive, but were unable to conclude on efficacy for other species of fin fish when used at this lower level. For these species other than salmonids, the ACAF concluded that the additive is only potentially efficacious at levels of 2,500 FTU/kg of Quantum® Blue.

2.3.1. Conclusions on efficacy

The ACAF concluded that Quantum Blue is efficacious in salmonids at an inclusion of 500 FTU/kg; however, they could not conclude on efficacy in other species of fin fish at this lower level. The additive has the potential to be efficacious at higher levels of 2,500 FTU/kg of Quantum® Blue in fin fish species other than salmonids.

3. Analytical methods evaluation

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for Quantum Blue (5 L, 10 L, 5 G, 40 P) (EURL, 2013):

> "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 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."

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical methods proposed as appropriate for official controls for this feed additive. The analytical methods were approved in 2013, at the time in which the UK was still an EU member.

4. Conclusions

The ACAF concluded that the additive was fully identified and characterised. Members concluded on a shelf-life of 12 months for the additive and on stability of the additive for 2 months in animal feed.

The additive is safe for the target animals, the consumer and the environment. It is not irritant to skin or eyes, is not a skin sensitiser, but should be treated as a potential respiratory sensitiser.

The ACAF concluded that Quantum® Blue is efficacious in salmonids at an inclusion of 500 FTU/kg; however, they could not conclude on efficacy in other species of fin fish at this level. The Committee concluded that the additive has the potential to be efficacious at higher levels of 2,500 FTU/kg in species of fin fish other than salmonids.

Abbreviations

Abbreviation	Definition
ACAF	Advisory Committee for Animal Feedingstuffs
CEN	Comité European de Noramalization
CFU	Colony forming units
DNA	Deoxyribonucleic acid
EC	European Commission
EURL	European Union Reference Laboratory
FCR	Feed conversion ratio
FSA	Food Standards Agency
FSS	Food Standards Scotland
FTU	Phytase activity
NOAEL	No observed adverse effect level
NOEL	No observed effect level

Abbreviation	Definition
TOS	Total organic solids

Acknowledgements

With thanks to the members of the ACAF during the course of the assessment, who were: Professor Nicholas Jonsson, Martin Briggs, Professor Emily Burton, Professor Katrina Campbell, Professor Matthew Fisher, Hannah Kane, Susan MacDonald, Dr. Oonagh Markey, Christine McAlinden, Dr. Donald Morrison, Derek Renshaw, Dr. Michael Salter, Dr. Adam Smith, Christel Wake, Dr. Helen Warren and Professor Nick Wheelhouse.

Published: May 29, 2025 BST.

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