REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment on the Safety and Efficacy of an Additive of 6-Phytase (VTR-Phytase) as a Feed Additive for All Avian Species and All Pigs (RP1026/1027)

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

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FSA Research and Evidence

An application was submitted to the Food Standards Agency in April 2021 from Victory Enzymes GmbH ('the applicant') for the new authorisation of an additive (VTR-phytase) containing 6-phytase, under the category 'zootechnical additives' and functional group 'digestibility enhancers'. The additive is proposed to be used at an inclusion rate of 500 U/kg of complete feed (12% moisture) for all avian species and all pigs.

To support the Food Standards Agency (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 characterised. No conclusion could be reached on the stability and homogeneity of the product. No further concerns were raised for Section II of the dossier.

The additive can be considered safe for the target species, consumers and the environment. The active substance is non-irritant to the skin and eyes, but the additive should be regarded as skin and respiratory sensitiser. Given the high dusting potential of the powder formulation, it is advised to limit user/worker exposure through inhalation.

VTR-phytase has the potential to be efficacious for all avian species, sows and pigs for fattening, and is efficacious in piglets when used at the proposed dose of 500 U/kg of complete feed with 12% moisture. Conclusions can be extrapolated to all *Suidae* at the corresponding developmental level.

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

1. Introduction

The FSA and FSS have undertaken an assessment for a feed additive of 6-phytase (VTR-phytase, Victory Enzymes GmbH, Fürschlag 3, D-91564, Neuendettelsau, Germany) under the assimilated Regulation (EC) No 1831/2003 (EC, 2003) in the category of 'zootechnical additives' and functional group 'digestibility enhancers', to favourably affect production in all avian species and all pigs. This is summarised within Table 1.

To support the safety assessment by 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 whether 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 ACAF at their February 2023 meeting, after which a request for information was communicated to the applicant. The response to this request was provided in April 2024 and was reviewed by the ACAF at their June 2024 meeting.

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 species or categories of animals
6-phytase produced by *Komagataella phaffii* GS115-VTR001	Feed additive	Zootechnical additive	All avian species and all pigs

2. Assessment

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

The additive's active substance is 6-phytase produced by the genetically modified strain of *Komagataella phaffii* GS115-VTR001. The additive is produced in two formulations, a solid containing a minimum enzymatic activity of 50,000 U/g and a liquid containing a minimum activity of 5,000 U/g. The applicant provided data from several batches supporting the identification values outlined below (Tables 2,3).

Table 2. Identity table for VTR-phytase solid form

Composition	
6-phytase activity	56,558 – 58,416 U/g
Water	4.94 – 7.45 %
Corn starch (carrier)	90.5-92.8 %
TOS	2.53 – 2.81 % w/w
Appearance	
Grey to white powder	
Chemical-physical specifications	
Dusting potential	4.98 – 7.90 g/m^3
Particle-size distribution	< 100 µm – 56.65 % < 50 µm – 48.81 % < 10 µm – 8.44 % < 1 µm – 0 %
Density	1.476 g/cm^3
Bulk density	0.679 g/cm^3
Impurities	
Arsenic	< 0.013 mg/kg
Lead	< 0.039 mg/kg
Cadmium	< 0.012 mg/kg
Mercury	< 0.003 mg/kg
Enterobacteriaceae	< 90 CFU/g
Yeasts	< 1,600 CFU/g
Moulds	< 200 CFU/g
Salmonella spp.	Absent in 25 g
Deoxynivalenol	< 3 µg/kg
Sum of Fumonisin B1+B2	< 7 µg/kg
Sum of HT2/T2	< 1.3 µg/kg
Zearalenone	< 0.7 µg/kg
Ochratoxin A	< 0.03 µg/kg
ndl-PCBs	< 0.3 ng/g
PCDD/Fs	0.0855 ± 0.022 pg WHO-TEQ/g
DLPCBs	0.0132 ± 0.003 pg WHO-TEQ /g
PCDD/Fs and DLPCBs	0.0987 ± 0.025 pg WHO-TEQ /g

The Committee was satisfied that the WGS presented in the application confirmed the production strain *Komagataella phaffii* GS115-VTR001 as a QPS microorganism. After a request for further information, the applicant provided satisfactory evidence of FAMI-QS certification for the production process. The Committee discussed the stability and homogeneity test reports presented, and noted a series of shortcomings in their design that would not allow them to conclude on their validity. The applicant was asked to repeat the stability and homogeneity studies. New data were presented following a significant delay from the time of the initial request for information. However, the reports submitted were incomplete and did

not include sufficient information to evaluate the new data. The Committee concluded that, based on the information presented, no conclusion could be reached on the stability and homogeneity of the additive.

Table 3. Identity table for VTR-phytase liquid form

Composition	
6-phytase activity	5,193 – 5,489 U/g
Water	89.5 – 90.2 %
TOS	0.22 – 0.23 % w/w
Appearance	
Yellow to light brown liquid	
Chemical-physical specifications	
Specific weight	1.08 ± 0.002 g/cm3
Viscosity	1.63 ± 0.03 mPas
Vapor pressure (25^{O}C and 40^{O}C)	2.24 kPa
Impurities	
Arsenic	< 0.005 mg/kg
Lead	< 0.001 mg/kg
Cadmium	< 0.00004 mg/kg
Mercury	< 0.002 mg/kg
Enterobacteriaceae	< 10 CFU/g
Yeasts	< 100 CFU/g
Moulds	< 100 CFU/g
Salmonella spp.	Absent in 25 g
Deoxynivalenol	< 3 µg/kg
Sum of Fumonisin B1+B2	< 7 µg/kg
Sum of HT2/T2 toxins	< 1.3 µg/kg
Zearalenone	< 0.7 µg/kg
Ochratoxin A	< 0.03 µg/kg

The proposed conditions of use of the additive are described in Table 4.

2.1.1. Conclusions on Section II

The ACAF concluded the additive was correctly characterised. No conclusion could be reached on the stability and homogeneity of the product. No further concerns were raised for Section II of the dossier.

Table 4. Conditions of use of VTR-Phytase proposed by the Applicant

Conditions of use proposed by the applicant (solid and liquid forms)				
Species or category of animal	Min-max Age	Min. content	Max. content	Withdrawal period
All avian species	--	500 U/kg feed	--	--
All pigs	--	500 U/kg feed	--	--
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use		Liquid form: Store refrigerated in original, closed packaging (<5°C). Powder form: Store in original, closed packaging, in cool, dry place (<25°C).		
Specific conditions or restrictions for handling		For user safety – Avoid direct contact and use in a well-ventilated area. Breathing protection and safety glasses are recommended.		
Post market monitoring		In compliance with EU feed hygiene legislation, traceability, HACCP, formal product/ service complaints procedure, and product recall capability.		
Specific conditions for use in complementary feedingstuffs		Final feeds must contain 10 g (solid) or 100 mL (liquid) per tonne feed.		

2.2. Section III: Safety

2.2.1. Safety for the target species and the consumer

The applicant presented results from two mutagenicity tests (a bacterial reverse mutation assay and an in vitro micronucleus test in cultured human lymphocytes) and a sub-chronic oral toxicity study in rats to support the safety of the additive for the target species. These studies were carried out on a liquid enzyme concentrate which included an average 6-phytase activity of 59,533 U/g.

Both genotoxicity studies were negative. The sub-chronic oral toxicity study showed no significant adverse effects at any of the doses tested up to 800 mg/kg bw/day, equivalent of 40,000 U/kg bw/day. Based on these results, the applicant proposed to establish 800 mg/kg bw/day as the NOAEL, and used the calculation proposed by EFSA in their guidance for the target species (FEEDAP, 2017) to derive a safe dose for the additive in several species. These values were extrapolated to physiologically related species, as referenced in Table 1 of the guidance:

- All poultry for fattening: 89 mg/kg feed (4,456 U/kg feed)
- Ornamental birds: 89 mg/kg feed (5,546 U/kg feed)
- All poultry for laying: 133 mg/kg feed (6,642 U/kg feed)
- All piglets: 160 mg/kg feed (8000 U/kg feed)
- All pigs for fattening: 192 mg/kg feed (9600 U/kg feed)
- Sows: 233 mg/kg feed (11667 U/kg)

The Committee accepted the study results and concluded that the additive can be considered safe for the target species.

The applicant used results from these toxicological studies to demonstrate safety of the additive for the consumer. The Committee accepted these conclusions and noted that, as the additive is an enzyme produced by a QPS organism, no further testing would be required.

2.2.2. Safety for the user/worker

Initially the applicant presented only one skin irritation test. After a request for further information, the following tests to evaluate the safety of the additive for the user/worker were presented:

- In vitro skin irritation study following OECD guideline 439 using EPISKIN™. carried out on the liquid concentrate formulation of the product.
- Two in vitro skin sensitisation studies following OECD guideline 442D carried out on the powder and liquid formulations of the product.
- Two in vitro skin sensitisation studies following OECD guideline 442E carried out on the powder and liquid formulations of the product.
- Two in vitro eye irritation studies following OECD guideline 492 carried out on the powder and liquid formulations of the product.

The Committee confirmed the applicant's conclusions that the active substance is non-irritant to the skin or eyes. The additive was shown to be a skin sensitiser. The additive is an enzyme-based product and all such products are considered to be respiratory sensitisers. Given the high dusting potential of the powder formulation, it is advised to limit user/ worker exposure through inhalation.

2.2.3. Safety for the environment

As an enzyme produced by a QPS microorganism, the additive is presumed to be safe for the environment. No further testing was carried out.

2.2.4. Conclusions on safety

The ACAF concluded that the proposed level of inclusion in feed is safe for the target species.

The active substance is non-genotoxic and the additive can be considered to be safe for the consumer and the environment.

The active substance is non-irritant to the skin and eyes, but the solid and liquid forms of the additive should be considered as skin and respiratory sensitisers. Given the high dusting potential of the powder formulation, it is advised to limit user/worker exposure through inhalation.

2.3. Section IV: Efficacy

2.3.1. Efficacy in all avian species

The applicant presented the following battery of studies to assess the efficacy of 6-phytase for all avian species:

- Short-term efficacy studies in laying hens:
    - Study 1: Doses of 500, 750 and 1000 U/kg, 10 replicates
    - Study 2: Doses of 500, 750 and 1000 U/kg, 10 replicates
    - Study 3: Doses of 500, 750 and 1000 U/kg, 10 replicates
- Long-term efficacy studies in broilers:
    - Study 4: Doses of 500, 750 and 1000 U/kg, 10 replicates
    - Study 5: Doses of 500, 750 and 1000 U/kg, 10 replicates
    - Study 6: Doses of 500, 750 and 1000 U/kg, 10 replicates

All six studies were carried out to a good quality standard and showed variable results across laying performance, egg quality and growth parameters. The effect of the additive in the measured parameters was significantly higher at the 750 and 1000 U/kg doses than the minimum proposed dose of 500 U/kg for both laying hens and broilers. The Committee concluded that VTR-phytase has the potential to be efficacious in laying hens and broilers at the minimum proposed dose of 500 U/kg of complete feed (12% moisture). Based on principles set out in the scientific guidance on the assessment of efficacy, conclusions from chickens for fattening and laying hens can be extrapolated to all avian species.

2.3.2. Efficacy in all pigs

The applicant presented the following battery of studies to assess the efficacy of 6-phytase for all porcine species:

- Short-term efficacy studies:
    - Study 1 in piglets: Doses of 500, 750 and 1000 U/kg, 8 replicates

    - Study 2 in sows: Doses of 500 U/kg, 8 replicates
    - Study 3 in sows: Doses of 500 U/kg, 6 replicates

- Long-term efficacy studies:

    - Study 4 in piglets: Doses of 500, 750 and 1000 U/kg, 48 replicates
    - Study 5 in piglets: Doses of 500, 750 and 1000 U/kg, 10 replicates
    - Study 6 in sows: Doses of 500, 750 and 1000 U/kg, 20 replicates

All six studies were carried out to a good quality standard and showed variable results across growth and reproductive parameters. The effect of the additive in the measured parameters was significantly better for piglets than in sows. The Committee concluded that VTR-phytase can be considered to be efficacious for piglets and that it has the potential to be efficacious in pigs for fattening and sows, when used at the minimum proposed level of 500 U/kg of complete feed (12% moisture). Conclusions can be extrapolated to all *Suidae* at the corresponding developmental level.

2.3.3. Conclusions on efficacy

The ACAF concluded that VTR-phytase has the potential to be efficacious for all avian species, pigs for fattening and sows, and that it is efficacious in piglets when used at the proposed dose of 500 U/kg of complete feed with 12% moisture. Conclusions can be extrapolated to all *Suidae* at the corresponding developmental level.

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 6-phytase (EURL-FA, 2021):

> "The Applicant proposed the ring-trial validated VDLUFA 27.1.4 and VDLUFA 27.1.3 methods for the quantification of the phytase activity, respectively, in the product (VTR-phytase) and premixtures and the ring-trial validated EN ISO 30024 method for the quantification of the phytase activity in feedingstuffs. Based on the performance characteristics available the EURL recommends for official control the ring-

> trial validated EN ISO and VDLUFA colorimetric methods mentioned above for the quantification of the phytase activity in the product, premixtures and feedingstuffs."

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.

4. Conclusions

The ACAF concluded the additive was correctly characterised. No conclusion could be reached on the stability and homogeneity of the product. No further concerns were raised for Section II of the dossier.

The ACAF concluded that the proposed level of inclusion in feed is safe for the target species.

The additive can be considered to be safe for the consumer and the environment.

The active substance is non-irritant to the skin and eyes, but the additive should be considered a skin and respiratory sensitiser. Given the high dusting potential of the powder formulation, it is advised to limit user/ worker exposure through inhalation.

The ACAF concluded that VTR-phytase has the potential to be efficacious for all avian species, sows and pigs for fattening, and that it is efficacious in piglets when used at the proposed dose of 500 U/kg of complete feed with 12% moisture. Conclusions can be extrapolated to all *Suidae* at the corresponding developmental level.

Abbreviations

Acronym	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
CFU	Colony forming units
EC	European Commission
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
FAMI-QS	Feed Additives and Pre-mixtures Quality System
FSA	Food Standards Agency
FSS	Food Standards Scotland
HACCP	Hazard Analysis and Critical Control Points
kPa	Kilopascal
mPas	Millipascal-second
Ndl-PCBs	Non-dioxin like polychlorinated biphenyls
NOAEL	No observed adverse effect level

Acronym	Definition
PCDD/Fs	Polychlorinated dibenzo-p-dioxins and dibenzofurans
DLPCBs	Dioxin-like polychlorinated biphenyls
OECD	Organisation for Economic Co-operation and Development
pg	Picogram
QPS	Qualified presumption of safety
TOS	Total Organic Solids
U	Enzyme units
VDLUFA	Association of German Agricultural Analytic and Research Institutes
WGS	Whole Genome Sequence

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