Assessment of the safety and efficacy of 6-phytase (Axtra® Phy Gold) as a feed additive for all poultry and pigs

Reference number RP420

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Regulated Product Dossier Assessment
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Abbreviations

Acronym	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
AFFAJEG	Animal Feed and Feed Additives Joint Expert Group
CAS	Chemical Abstracts Service
CFU	Colony forming units
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EURL	European Union Reference Laboratory
FSA	Food Standards Agency
FSS	Food Standards Scotland
FTU	Phytase units
LOQ	Limit of quantification
MSDS	Material Safety Data Sheet
NOAEL	No observed adverse effect level
OECD	Organisation for Economic Cooperation and Development

Summary

An application was submitted to the Food Standards Agency in February 2021 from Genencor International B.V. (“the applicant”) for the authorisation of an additive (6-phytase - Axtra® Phy Gold), under the category of ‘zootechnical additives’, functional group ‘digestibility enhancers’ for its use in all poultry and pigs.

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

The Animal Feed and Feed Additives Joint Expert Group (AFFAJEG) and the Advisory Committee on Animal Feedingstuffs (ACAF) concluded that the additive was correctly identified and characterised, and that the additive can be considered safe for the target species, the consumer and the environment. The additive should be considered a respiratory sensitiser. The additive is non-irritant to eyes and skin, and it is not a skin sensitiser.

Based on data from 12 efficacy trials in broilers, laying hens, sows and piglets, the Committee concluded that Axtra® Phy Gold, in its three formulations (30L, 30T and 65G), can be considered efficacious in all poultry and pigs at the proposed minimum dose of 300 phytase units (FTU)/kg of complete feed (laying birds) and 500 FTU/kg of complete feed (other target species).

The views of AFFAJEG and 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 a risk assessment for a feed additive (6-phytase - Axtra® Phy Gold, Genencor International B.V., Willem Einthovenstraat 4, 2342 BH, Oegstgeest, The Netherlands) under retained regulation (EC) No 1831/2003[1], under the category 'zootechnical additives', functional group 'digestibility enhancers' for its use in all poultry and pigs.

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

The dossier was evaluated on behalf of the FSA and FSS by the AFFAJEG and the ACAF. 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.

With thanks to the members of the AFFAJEG and ACAF during the course of the assessment, who were: Professor John Wallace, Professor Nicholas Jonsson, Martin Briggs, Professor Katrina Campbell, Susan MacDonald, Professor Matthew Fisher, Christine McAlinden, Dr. Donald Morrison, Derek Renshaw, Dr. Michael Salter, Dr. Adam Smith, Dr. Helen Warren and Dr. Nick Wheelhouse.

The dossier was evaluated by the AFFAJEG at their April 2022, and by ACAF at their October 2022 and April 2023 meetings. Further information was provided by the applicant in December 2021, August 2022 and February 2023 responding to queries by the FSA.

This document outlines the discussion and conclusions of the AFFAJEG's and ACAF's assessment on the safety and efficacy of Axtra® Phy Gold as a feed additive.

2. Assessment

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

The additive is a preparation containing 6-phytase, produced by a genetically modified strain of *Trichoderma reesei*, with three formulations proposed:

- 30L: Liquid, with a minimum activity of 30,000 phytase units (FTU)/g
- 30T: Granular, minimum activity of 30,000 FTU/g
- 65G: Granular, minimum activity of 65,000 FTU/g

The applicant provided data from several batches supporting the identity values outlined below.

Table 1: Identity table Axtra® Phy Gold 30L/30T/65G

Axtra® Phy Gold 30L	
Composition	
6-phytase	Minimum activity of 30,000 FTU/g
Appearance	
Brown liquid	
Chemical-physical characteristics	
Viscosity	9-10 mPa*s at 25°C
Axtra® Phy Gold 30T	
Composition	
6-phytase	Minimum activity of 30,000 FTU/g
Appearance	
Off-white to light tan, fine granular product	
Chemical-physical characteristics	
Dusting potential	Average of 7 mg/m^3 of air
Particle size distribution	Mean diameters for different batches were 408 – 448 µm; 90% of particles with diameter < 600 µm; absence of particles < 228 µm
Axtra® Phy Gold 65G	
Composition	
6-phytase	Minimum activity of 65,000 FTU/g
Appearance	
Off-white to light tan, fine granular product	
Chemical-physical characteristics	
Dusting potential	Average of 13 mg/m^3 of air
Particle size distribution	Mean diameters of different batches were 325 –

	342 µm; 90% of particles with diameter < 430 µm; absence of particles < 200 µm
Axtra® Phy Gold 30L, 30T and 65G	
Microbiological profile	
Salmonella	Absent in 25 g of sample
E. coli	Absent in 25 g of sample
Total coliforms	< 30 CFU/g
Antimicrobial resistance	Negative
Heavy metals	
Lead	Not more than 5 mg/kg

Several of the tests presented for Section II were carried out on a concentrate version of the product. Upon evaluation of the equivalence of substances, the ACAF concluded that the tests could be considered representative for the final form of the product.

The ACAF identified several items of information that the applicant would have to provide in order to inform the assessment of the product, including updated certificates, and provision of material safety data sheets (MSDS) for all substances used in the production process. These queries were addressed by the applicant.

The stability of the additive under conditions of high heat for several minutes was questioned by the Group, in answer to which the applicant presented further evidence, showing the additive is stable for up to 2 minutes at 82°C.

The applicant proposes that the additive is included in animal feed as shown in Table 2:

Table 2: Proposed mode of use of Axtra® Phy Gold (30L, 30T, 65G)

Proposed mode of use in animal nutrition		
Additive	30,000 FTU/g 6-phytase (30L, 30T) 65,000 FTU/g 6-phytase (65G)	
CAS number	9001-89-2	
Category and functional group	Zootechnical additive, digestibility enhancer	
Description		
Composition, description	**Formula**	**Method of analysis**
Preparation of 6-phytase (EC 3.1.3.26) produced by *Trichoderma reesei*	Minimum 6-phytase activity (30L, 30T): 30,000 FTU/g Minimum 6-phytase activity (65G): 65,000 FTU/g	Colourimetric method
Trade name		Axtra® Phy Gold (30L, 30T, 65G)
Name of the holder of authorisation		Genencor

			International B.V.
Conditions of use			
Species or category of animal	**Maximum Age**	**Minimum content of 6-phytase in complete feed**	**Withdrawal period**
All avian species for fattening	To slaughter age and weight	500 FTU/kg	N/A
All avian species reared for laying and for breeding	No maximum age	500 FTU/kg	
Laying hens	From point of lay	300 FTU/kg	
Piglets (weaned)	Up to 35 kg	500 FTU/kg	
Pigs for fattening	To slaughter age and weight	500 FTU/kg	
Sows	-	500 FTU/kg	
Minor growing porcine species	To slaughter age and weight	500 FTU/kg	

2.1.1. Conclusions on Section II

The ACAF concluded that the additive was correctly identified and characterised.

No further concerns were raised for Section II of the dossier.

2.2. Section III: Safety

2.2.1. Safety for the target species

No tolerance studies were provided. Evidence of safety for the target species was addressed through toxicological studies with oral administration carried out in laboratory animals.

The applicant presented a 90-day repeated dose toxicity study administered by oral gavage in rats to evaluate the safety of the additive for the target species. Rats were administered doses of 250, 500 and 1000 mg/kg bw/day of enzyme concentrate (the dose levels were expressed as the amount of total organic solids). The study was conducted in accordance with OECD Test Guideline 408 and showed no adverse effects at any of the doses tested. The NOAEL was agreed to be the highest dose tested of 1000 mg/kg bw/day (equivalent to 450,000 FTU/kg bw/day). Comparing the proposed conditions of use of the additive, there are margins of safety of at least 100 times the 500 FTU/kg intended dose for all target species except laying avian species, and 249 times the 300 FTU/kg dose for laying avian species.

The ACAF concluded that the additive is safe for the target species at the recommended doses.

2.2.2. Safety for the consumer

Three studies were presented to evaluate the safety of the additive:

- A bacterial reverse mutation assay following OECD 471.
- An *in vitro* mammalian cell micronucleus test.
- A 90-day repeated dose oral toxicity study in rats following OECD 408.

No adverse effects were observed in the bacterial reverse mutation assay or the 90-day toxicity study. The Committee noted that the *in vitro* mammalian cell micronucleus test had not used the recommended aneugen positive controls as detailed in OECD guideline 487, and requested the applicant to repeat the test. The new test provided used appropriate positive and negative aneugen controls, and the Committee concluded that no adverse effects were shown, and that the additive is non-genotoxic. Residue studies and exposure assessment are not required for feed additives composed of enzymes.

Based on the information presented in the dossier, the ACAF concluded that the additive is safe for consumers.

2.2.3. Safety for the user

The additive is proteinaceous in nature and is, therefore, regarded as a respiratory sensitiser by default. Measures should be taken to minimise worker exposure by inhalation. A skin irritation test following OECD 439, an eye irritation test following OECD 492 and a local lymph node assay in mice were presented. Upon evaluation of the test reports presented, ACAF concluded that the additive is non-irritant to eyes and skin and that it is not a skin sensitiser.

2.2.4. Safety for the environment

The active substance of the additive is a protein, and as such it is expected to be degraded in the digestive tract of the target animals. The ACAF concluded that the additive can be considered safe for the environment.

2.2.5. Conclusions on safety

The AFFAJEG concluded that the additive can be considered safe for the target species, the consumer and the environment.

The additive should be considered a respiratory sensitiser. The additive is non-irritant to eyes and skin, and it is not a skin sensitiser.

2.3. Section IV: Efficacy

The applicant presented a battery of efficacy trials. Table summaries for the studies can be found in Appendix 1:

- Study 1, 108 chickens for fattening, 11 days, table 3.
- Study 2, 320 chickens for fattening, 21 days, table 4.
- Study 3, 360 chickens for fattening, 21 days, table 5.
- Study 4, 180 laying hens, 28 days, table 6.
- Study 5, 216 laying hens, 28 days, table 7.
- Study 6, 384 laying hens, 23 weeks, table 8.
- Study 7, 30 sows, 18 days, table 9.
- Study 8, 30 sows, 18 days, table 9.
- Study 9, 30 sows, 20 days, table 9.
- Study 10, 24 weaned piglets, 12 days, table 10.
- Study 11, 16 weaned piglets, 11 days, table 10.
- Study 12, 27 weaned piglets, 11 days, table 11.

The ACAF evaluated the studies and determined that their design and execution was satisfactory. No further concerns were raised. The Group concluded that the additive can be considered efficacious in the target species at the proposed doses of 500 and 300 FTU/kg of complete feed.

2.3.1. Conclusions on Efficacy

The additive can be considered efficacious for all poultry and pigs at the proposed doses of 500 and 300 FTU/kg of complete feed.

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 analysis for 6-phytase[2]:

"The Applicant submitted single-laboratory validated and further verified methods for the quantification of the *phytase* activity in the *product (Axtra® PHY GOLD)*, *premixtures* and *feedingstuffs*. The submitted methods are very similar to the ring-trial validated EN ISO 30024 method.

Upon request of the EURL, the Applicant compared both protocols confirming that equivalent results are obtained when applying the slightly different methods to *feedingstuffs* containing the product (*Axtra® PHY GOLD*).

Additionally the EURL is aware of other ring-trial validated VDLUFA methods specifically describing the preparation of *premixtures* (VDLUFA 27.1.3) and *feed additives* (VDLUFA 27.1.4) for the quantification of their *phytase* activity according to EN ISO 30024.

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*.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary."

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The AFFAJEG/ACAF concluded that the additive was correctly identified and characterised.

The AFFAJEG/ACAF concluded that the additive can be considered safe for the target species, the consumer and the environment.

The additive should be considered a respiratory sensitiser. The additive is non-irritant to eyes and skin, and it is not a skin sensitiser.

The additive can be considered efficacious for all poultry and pigs at the proposed doses of 500 and 300 FTU/kg of complete feed, if adequate phytate is present in the diet.

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

5. References

1. EC (European Commission), 2003. Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition. Available at https://www.legislation.gov.uk/eur/2003/1831/contents

2. EURL-FA (European Reference Laboratory for Feed Additives), 2021. Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003. Preparation of 6-phytase. Available at: finrep_fad-2020-0083_axtra-phy-gold.pdf (europa.eu)

6. Appendix 1

Table 3: Study 1. Efficacy of Axtra® Phy Gold in broilers

Treatment	Treatment code	N	ADFI (g)	ADWG (g)	FCR (g/g)	Coefficient of P retention (%) mean	Retainable P (g/kg) mean
Mash feed							
Positive control	T1	9	70.0	51.5	1.361	50.70^{b}	3.09^{a}
Negative control	T2	9	61.1	41.8	1.466	44.3^{c}	1.82^{c}
NC + 500 FTU/kg	T4	9	70.9	51.4	1.388	71.2^{a}	2.92^{ab}
Pelleted feed							
Positive control	T6	9	84.6	64.7	1.310	46.4^{bc}	2.83^{b}
Negative control	T7	9	79.1	56.7	1.393	48.0^{bc}	1.97^{c}
NC + 500 FTU/kg	T9	9	89.0	68.8	1.293	66.3a	2.72^{b}
Feed							
Mash		27	67.3^{b}	48.2^{b}	1.405	55.4	2.61^{a}
Pellet		27	84.2^{a}	63.4^{a}	1.332^{b}	53.6	2.51^{b}
Diet							
Positive control		18	77.3^{a}	58.1^{a}	1.335^{b}	48.5^{b}	2.96^{a}
Negative control		18	70.1^{b}	49.3^{b}	1.429^{a}	46.1^{b}	1.89^{c}
NC + 500 FTU/kg		18	79.9a	60.1^{a}	1.341^{b}	68.8^{a}	2.82^{b}

N – Number of replicates; ADFI – average daily feed intake; ADWG – average daily weight gain; FCR – feed conversion ratio (feed:gain); P - phosphorus. Different superscripts within a column indicate a statistically significant ($P<0.05$) difference, Tukey's multiple range test.

Table 4: Study 2. Effect of Axtra® Phy Gold in broilers

Treatment	ADFI (g/b/d)	ADG (g/b/d)	FCR (g:g)	CaR	PR	Ca excreta (g/kg DM)	P excreta (g/kg DM)
Positive control	45.6[a]	30.1[a]	1.519[a]	0.414[a]	0.577[a]	22.7[a]	12.4[a]
Negative control	40.1[b]	21.8[b]	1.855[b]	0.172[b]	0.439[b]	17.9[b]	8.2[b]
NC + 500 FTU/kg	45.2[a]	28.5[a]	1.591[a]	0.375[a]	0.626[a]	17.6[b]	7.1[bc]
NC + 1000 FTU/kg	45.8[a]	30.3[a]	1.512[a]	0.451[a]	0.676[a]	15.4[b]	6.2[c]
SEM	0.81	0.82	0.034	0.0395	0.0284	0.78	0.28
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

ADFI – average daily feed intake; ADG – average daily gain; FCR – feed conversion ratio (feed:gain); CaR/PR – coefficient of retained dietary calcium and phosphorus; SEM – standard error of mean. Different superscripts within a column indicate a statistically significant (P<0.05) difference.

Table 5: Study 3. Efficacy of Axtra® Phy Gold in broilers

Treatment	FI (g)	BWG (g)	FCR (g/g)	ret. P (%)	ex. P(%)	Tibia DM (%)	Defatted tibia ash (%, DM basis)
Positive control	1264.08[a]	985.75[a]	1.28[c]	54.43[b]	1.02[a]	44.45[a]	49.28[a]
Negative control	1145.75[b]	846.0[b]	1.35[a]	55.70[b]	0.80[b]	40.72[b]	44.48[c]
NC + 500 FTU/kg	1262.25[a]	956.42[a]	1.32[b]	66.44[a]	0.58[c]	43.67[a]	47.27[b]
SEM	17.74	0.82	0.034	1.75	0.031	0.41	0.42
P-value	<0.0001	<0.0001	<0.0001	<0.001	<0.001	<0.001	<0.001
N	12	12	12	12	12	12	12

FI – feed intake; BWG - body weight gain; FCR – feed conversion ratio (feed:gain); P – phosphorus; ret- retention; ex- excretion; DM – dry matter; SEM – standard error of mean; N- No. replicates per treatment; Different superscripts within a column indicate a statistically significant (P<0.05) difference. Birds were fed the treatment diets from d7 to 21, the performance were measured for the whole period of 0-21 days.

Table 6: Study 4. Efficacy of Axtra® Phy Gold in laying hens

Treatment	Egg P (%)	P ret (%)	Ca ret (%)
Positive control	0.204	31.71^{c}	64.33^{bc}
Negative control	0.189	27.25^{d}	61.07^{c}
NC + 300 FTU/kg	0.195	35.59^{b}	67.20^{ab}
NC + 600 FTU/kg	0.198	38.08^{a}	70.05^{a}
NC + 900 FTU/kg	0.202	38.78^{a}	70.81^{a}
SEM	0.005	0.555	1.067
P-value	0.326	<0.001	<0.001
P ret – phosphorus retention; Ca ret – calcium retention; SEM – standard error of mean; Different superscripts within a column indicate a statistically significant (P<0.05) difference.			

Table 7: Study 5. Efficacy of Axtra® Phy Gold in laying hens

Treatment	Egg P (%)	P ret (%)	Ca ret (%)
Positive control	0.172	25.14^{b}	38.54
Negative control	0.173	25.54^{b}	43.37
NC + 300 FTU/kg	0.176	36.11^{a}	42.24
SEM	0.004	2.69	2.90
P-value	0.73	0.29	0.0017
P ret – phosphorus retention; Ca ret – calcium retention; SEM – standard error of mean; Different superscripts within a column indicate a statistically significant (P<0.05) difference.			

Table 8: Study 6. Efficacy of Axtra® Phy Gold in laying hens

Treatment	P ret (%)	Ca ret (%)
Negative control	19.18[b]	64.15[b]
NC + 300 FTU/kg	28.88[a]	66.13[a]
SEM	1.284	0.681
P-value	<0.001	0.046

P ret – phosphorus retention; Ca ret – calcium retention; SEM – standard error of mean; Different superscripts within a column indicate a statistically significant (P<0.05) difference.

Table 9: Studies 7, 8 and 9. Efficacy of Axtra® Phy Gold in sows

Treatment	P digestibility (%)	Ca digestibility (%)
Study 7		
Positive control	32.4[b]	25.3[a]
Negative control	27.9[a]	32.1[ab]
NC + 500 FTU/kg	44.3[c]	35.3[b]
SEM	0.89	1.96
P-value	<0.001	<0.01
Study 8		
Positive control	28.8[b]	20.8
Negative control	19.7[a]	17.9
NC + 500 FTU/kg	34.2[c]	23.9
SEM	1.50	1.99
P-value	<0.001	0.07
Study 9		
Positive control	34.25[a]	28.13[b]
Negative control	27.83[b]	29.13[b]
NC + 500 FTU/kg	35.37[a]	36.99[a]
SEM	1.53	2.35
P-value	0.003	0.014
P – phosphorus; Ca– calcium; SEM – standard error of mean; Different superscripts within a column indicate a statistically significant (P<0.05) difference.		

Table 10: Studies 10 and 11. Efficacy of Axtra® Phy Gold in piglets

Treatment	ATTD (%)		Retention (%)	
	Ca	P	Ca	P
Study 10				
Negative control	43.9[a]	65.9[a]	-	43.18[a]
NC + 500 FTU/kg	56.4[b]	72.4[b]	-	55.26[b]
NC + 1000 FTU/kg	68.1[c]	78.0[b]	-	67.09[c]
SEM	2.286	1.467	-	2.261
P-value	<0.001	<0.001	-	0.001
Study 11				
Negative control	58.91[a]	42.05[a]	44.81[a]	41.36[a]
NC + 500 FTU/kg	74.76[b]	66.84[b]	64.68[b]	66.11[b]
SEM	2.780	3.615	3.500	3.606
P-value	<0.001	<0.001	<0.001	<0.001
ATTD – apparent total tract digestibility; Ca– calcium; P – phosphorus; SEM – standard error of mean; Different superscripts within a column indicate a statistically significant (P<0.05) difference.				

Table 11: Study 12. Efficacy of Axtra® Phy Gold in piglets

Treatment	Digestibility co-efficient			Retention co-efficient		
	DM	Ca	P	DM	Ca	P
Negative control	0.91	0.81	0.64[a]	0.83[a]	0.68[a]	0.60[a]
NC + 500 FTU/kg	0.92	0.85	0.77[b]	0.83[a]	0.75[ab]	0.73[b]
NC + 1000 FTU/kg	0.93	0.85	0.79[b]	0.87[b]	0.78[b]	0.77[b]
SED	0.009	0.033	0.047	0.016	0.037	0.046
P-value	0.147	0.303	0.010	0.043	0.051	0.005
DM – dry matter; Ca– calcium; P – phosphorus; SED – standard error of difference; Different superscripts within a column indicate a statistically significant (P<0.05) difference.						

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