Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed and the Scientific Panel on Genetically Modified Organisms on the safety and efficacy of the enzymatic preparation Phyzyme XP (6- Phytase) for use as feed additive for chickens for fattening

(Question No EFSA-Q-2005-063)

Adopted by
the FEEDAP Panel on 20 April 2006
and by
the GMO Panel on 28 February 2006

SUMMARY

Phyzyme XP is an enzyme feed additive with 6-phytase as its main activity, produced after fermentation with the genetically modified yeast *Schizosaccharomyces pombe,* designated ASP595-1 and deposited in the American Collection of Type Cultures as strain ATCC 5233. The final production strain is obtained after transformation with a linearised DNA fragment containing eight tandem repeats of an expression cassette which consists of the cytomegalovirus promoter, the 5' untranslated region of human lipocortin1 cDNA, the 6-phytase open reading frame, the terminator and the 3' untranslated region of human lipoprotein1 cDNA. No antibiotic resistance marker sequences were co-transformed as was confirmed by PCR. The expression cassettes are integrated in the *leu1* gene of the *S. pombe* chromosome. The applicant provided an acceptable risk analysis of the introduced viral sequences. Thus, no harmful sequences have been introduced, nor are there any antibiotic resistance marker sequences in the final production strain.

The safety of the donor and recipient organisms is based on the concept of familiarity.

After fermentation the enzyme is purified and the final enzyme product contains no culturable production organisms and recombinant DNA is below the level of detection.

Three pen trials conducted over the 42-day growing period showed significant effects on feed efficiency, daily weight gain and/or feed intake with the supplementation of 500 FTU Phyzyme XP kg^{-1} feed. P digestibility was also demonstrated to be increased with 500 FTU Phyzyme XP kg^{-1} feed. Only two studies were provided with supplementation of diets with 250 FTU Phyzyme XP kg^{-1} feed, both showing significant effects on weight gain, feed conversion and feed intake in two studies and on bone ash in one study. Therefore it is concluded that the efficacy of Phyzyme XP has been demonstrated at the dose of 500 FTU kg^{-1}, but insufficient data is provided to demonstrate efficacy at the minimum recommended dose of 250 FTU kg^{-1}.

A tolerance test carried out with chickens for fattening over a period of six weeks with the recommended dose (500 FTU kg^{-1}) and 7.5-fold overdose (7500 FTU kg^{-1}) of Phyzyme XP produced no evidence of any adverse effect on performance or mortality. Therefore it is concluded that the safety of Phyzyme XP has been demonstrated at the recommended dose and the 7.5-fold overdose.

On the basis of the results of three genotoxicity studies and one 90-day toxicity study there is no concern regarding the safety of this product for the consumer.

The product showed slight evidence of irritancy, low acute inhalation toxicity and no evidence of dermal sensitisation. By convention, the product, being an enzyme, is considered as a potential respiratory sensitizer.

It is concluded that there is no evidence for concern regarding safety for the environment.

Keywords: Enzyme, phytase, chickens for fattening, efficacy, safety, *Schizosaccharomyces pombe*, genetically modified micro-organism.

BACKGROUND

Regulation (EC) No 1831/2003 establishes rules governing the Community authorisation of additives for animal nutrition and in particular defines the conditions that a substance/product should meet to be granted authorisation. This Regulation replaces Council Directive 70/524/EEC. The Regulation foresees transition procedures for handling applications submitted under the earlier directive in its Article 25.

The applicant company Danisco Animal Nutrition is seeking permanent Community authorisation of its enzyme product "Phyzyme XP", which is a preparation of 6-Phytase EC 3.1.3.26, produced by *Schizosaccharomyces pombe* (ATCC 5233), as a feed additive intended for use in chickens for fattening under category "Enzymes" (Table 1).

Table 1. **Description of the preparation**

Product category:	Enzyme
Trade name:	Phyzyme XP
Description:	6-Phytase EC 3.1.3.26, produced by *Schizosaccharomyces pombe* ATCC 5233
Target animal category:	Chickens for fattening
Applicant:	Danisco Animal Nutrition
Member State Rapporteur:	UK
Type of request:	Permanent authorisation

In accordance with the above legislation, the Commission received a request from this company, through UK, the Member State *rapporteur* chosen by the company, to authorize its product under the conditions set out in Table 2. Supporting data produced by the company were compiled in a dossier and accompanied that application. All Member States received the dossier, which was introduced at the Standing Committee on the Food Chain and Animal Health. This dossier has been made available to the European Food Safety Authority.

TERMS OF REFERENCE

In view of the above, the Commission asks the European Food Safety Authority to deliver an opinion on the efficacy and on the safety of the enzyme preparation of trade name "Phyzyme XP", a preparation of 6-phytase produced by *Schizosaccharomyces pombe* (ATCC 5233), for the consumer, the user, the workers and for the target animal category specified and the environment, when used under the conditions set out in Table 2 below, taking into account the background and the information submitted by the applicant in the dossier.

Table 2: **Annex entry proposal**

Additive	Chemical formula, description	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions
				Units of activity kg^{-1} of complete feedingstuff		
Phyzyme XP EC 3.1.3.26	Preparation of 6-Phytase produced by *Schizosaccharomyces pombe* (ATCC 5233) having a minimum activity of: Liquid form: 5000 FTU[11] g^{-1}	Chickens for fattening	-	250 FTU	-	1. In the directions for use of the additive and premixture, indicate the storage temperature, storage life and stability to pelleting. 2. Recommended dose per kg of complete feedingstuff: 250 - 1000 FTU. 3. For use in compound feed containing more than 0.23% phytin bound phosphorus

[1] FTU is the amount of enzyme which liberates 1 micromole of inorganic phosphate per minute from sodium phytate substrate at pH 5.5 and at 37 °C

ASSESSMENT

1. Introduction

The subject of the present dossier is the permanent authorisation of Phyzyme™ XP liquid for chickens for fattening. This product is a preparation of 6-phytase produced by the genetically modified micro-organism (GMM) *Schizosaccharomyces pombe* (ATCC 5233). It is intended to be used as an additive to increase the bioavailability of phosphorus from the diet by hydrolysing the plant phytate. This would reduce the need to add inorganic phosphorus supplements to the animal diets and would decrease the excretion of total phosphorus in the manure.

2. Characterisation

2.1. Characterisation of the product

Phyzyme™ XP is an enzyme feed additive with 6-phytase as its main activity. The production strain, designated ASP595-1, is derived from the fission yeast *Schizosaccharomyces pombe* (ATCC 38399).

The product is a liquid enzyme concentrate complying with the following specifications:

Appearance	Homogeneous brown liquid
Specific gravity (g mL^{-1})	1.05 - 1.15
pH	4.4 – 4.8
Phytase activity (units g^{-1})	5000 guaranteed minimum
Sorbitol (% w v^{-1})	4.0%
Sodium Chloride (% w v^{-1})	13%

Units: µmoles phosphate per min at pH 5.5 and 37ºC

Routine quality control requires examination for microbiological (coliforms, *E. coli*, *Salmonella* spp., yeasts and molds), heavy metals (arsenic, cadmium, lead and mercury) and mycotoxin contamination. Appropriate action limits are set.

The product has been demonstrated to be stable on storage with 96% of the enzyme activity retained after 6 months at 5ºC, 92% after 6 months at 20ºC and 76% after 6 months at 35ºC. Phyzyme™ XP is also stable after mixing with feedingstuffs with approximately 80% of activity remaining after 6 months storage at 20ºC and 35ºC.

2.2. Characterisation of the production organism

2.2.1. Information relating to the genetically modified micro-organism (GMM)

2.2.1.1. Characteristics of the recipient or parental micro-organism

The recipient strain is *Schizosaccharomyces pombe* ATCC 38399, a teleomorph heterothallic$^-$ leucine auxotrophic yeast strain. The leucine auxotrophy results from a mutation at the leucine locus *leu1-32*.

S. pombe has a long history of safe use (OECD, 1992; IFBC, 1990). It is present in various traditionally fermented foods.

Schizosaccharomyces are members of the ascomycete subgroup Archaeascomycetes, a recently described but not yet formalized group designated primarily upon rDNA sequence analysis. The genus *Schizosaccharomyces* contains three recognized species: *S. japonicus*, *S. ocotsporus,* and *S. pombe. S. pombe* is taxonomically distinct from the most common recognized yeast pathogens. There are no reports of the existence of pathogenic, toxigenic or allergenic strains in the genus *Schizosaccharomyces* nor has any strain been described as being isolated from human faeces or from clinical samples derived from the human body.

2.2.1.2. Characteristics of the donor organism

The donor organism is *Escherichia coli* B ATCC 11303. The DNA was supplied by Sigma Co. The origin of the strain is not known. It was isolated in the late 1930's or early 1940's and since then it has been used as a common laboratory strain.

Escherichia coli is a well known species belonging to the family *Enterobacteriaceae*.

E. coli is found throughout the world in water, soil and as intestinal flora. Certain *E. coli* strains have pathogenic potential. Safety of the *E. coli* B strain is based on 1) the long-term use of this organism in numerous laboratories throughout the world, 2) the absence of genes encoding virulence factors as determined by PCR and other molecular methods, and 3) the lack of pathogenic potential in both mouse and chick models.

2.2.1.3. Description of the genetic modification process

The open reading frame (ORF) of the *appA* 6-phytase gene without its native *E. coli* leader peptide sequence was amplified and ligated in an expression vector. The nucleotide sequence of the insert was determined in its entirety to confirm that no mutations had been introduced. The expression cassette, which consists of the cytomegalovirus promoter, the 5' untranslated region of human lipocortin1 cDNA, the 6-phytase ORF, the terminator and the 3' untranslated region of the gene encoding human lipoprotein1, was religated so that eight expression cassettes were constructed as tandem repeats. The eight tandem expression cassettes were digested from the plasmid and the linearised DNA fragment separated from the plasmid fragment encoding ampicillin resistance. They were then transferred into *S. pombe* in two rounds of gene replacement recombination using auxotrophic mutants and selection for prototrophy. The strain producing the highest quantity of phytase was used as the final production strain. The copy number of the expression cassette was determined by quantitative PCR during the scaling up production process and varied from 4.35 ± 0.27 to 2.34 ± 0.22.

2.2.2. Information relating to the production process

Phyzyme™ XP is produced by a contained system of submerged, fed-batch pure culture fermentation of the genetically modified strain *S. pombe*. The 6-phytase enzyme is recovered from the fermentation broth by physical operation. The production process is controlled by process control parameters (media composition, sterilization, temperature, agitation, aeration, pressure, growth duration, pH, residual glucose level) and the fermentation process is followed by quality control parameters (culture purity, growth, phytase activity).

2.2.3. Information relating to the product purification process

2.2.3.1. Technique used to remove microbial cells from the product

A number of process steps serve to prevent the presence of the production organism in the final product. In the cell separation step, using centrifugation or rotary vacuum filtration, the majority of yeast cells (*i.e.*, > 95%) are removed. In the clarification step, either conventional (depth) filtration or tangential flow microfiltration ensures that a cell-free process stream is provided for the ultrafiltration step. The polish filtration step is an additional conventional (depth) filtration or tangential flow microfiltration step, which further ensures that no production organism is present in the final product. The absence of viable yeast cells is tested in the final product by plating reaching a detection level of 2 cfu ml^{-1}. Dot blot analysis was used to confirm that recombinant DNA was absent from the enzyme product. The limit of detection in this assay was 300 pg DNA kg^{-1} feed. The GMO Panel accepts the detection limit of this method as being adequate.

3. Efficacy for chickens for fattening

Four experiments have been carried out in order to demonstrate the efficacy of this enzyme preparation in chickens for fattening.

3.1. Trial 1

One pen trial with 1512 chickens for fattening was performed from 1 to 42 days (1 - 21 days = starter period, 21 – 42 days = finisher period). The chicks were allocated into 36 pens, in a randomised design, consisting of six dietary treatments with six replicates per treatment. The basal diet contained 5.16/4.50 g kg^{-1} total P (starter/finisher diet, respectively). Phyzyme XP was tested at 0, 250, 500, 750 and 1000 U kg^{-1}. For comparison, a positive control containing 6.35/5.69 g kg^{-1} total P was included. Enzyme activity in feed was measured and found appropriate.

The addition of Phyzyme XP at 250 FTU kg^{-1} resulted in a significant ($P \leq 0.05$) increase in body weight gain and feed efficiency during the study (Table 3). The highest improvement in feed efficiency was observed for the 500 FTU kg^{-1} group. Mortality was not affected by treatment.

Table 3. **Performance of chickens for fattening (1-42 days)**

Phyzyme XP (FTU kg^{-1})	Weight gain (g)	Feed intake (g $bird^{-1}$)	Feed conversion (gain/feed)	Mortality (%)
Positive control	2117^{b}	3780	0.553^{bc}	4.36
0	1939^{a}	3688	0.516^{a}	5.16
250	2126^{b}	3847	0.544^{b}	7.14
500	2160^{b}	3827	0.558^{c}	7.14
750	2195^{b}	3862	0.559^{c}	3.57
1000	2138^{b}	3819	0.555^{bc}	5.95

$^{a, b, c:}$ Means in a column not sharing a common superscript are significantly different ($P<0.05$)

3.2. Trial 2

One pen trial with 480 chickens for fattening from 1 to 42 days (1 - 21 days = starter period, 21 – 42 days = finisher period) was performed. The chicks were allocated into 40 pens, in a randomised design, consisting of 5 dietary treatments with 8 replicates per treatment.

The basal diet contained 5.1/4.4 g kg^{-1} total P (starter/finisher diet). Phyzyme XP was tested at 0, 500, 750 and 1000 FTU kg^{-1}. For comparison a positive control diet was used which contained 7.7/6.4 g kg^{-1} total P. Enzyme activity in feed was measured and found appropriate. At the end of the 3rd and 6th weeks, two chicks from each pen were killed and the left tibia and toe from each were excised for bone ash analysis.

Table 4. **Performance of chickens for fattening fed diets with graded levels of Phyzyme XP (1-42 days)**

Phyzyme XP (FTU kg^{-1})	Weight gain (g)	Feed intake (g $bird^{-1}$)	Feed conversion (gain/feed)	Mortality (%)	Ash (%)	
					Tibia	Toe
Positive control	2198^{a}	3723^{a}	0.591	4.16	54.3^{a}	12.8^{a}
0	2005^{b}	3430^{b}	0.585	6.25	48.5^{b}	10.9^{b}
500	2134^{ab}	3674^{a}	0.581	3.13	51.9^{a}	12.2^{ab}
750	2246^{a}	3797^{a}	0.591	4.17	52.7^{a}	12.5^{a}
1000	2209^{a}	3740^{a}	0.591	5.21	52.0^{a}	11.7^{ab}

$^{a, b:}$ Means in a column not sharing a common superscript are significantly different ($P<0.05$)

Supplementation of the diet with Phyzyme XP at 500 FTU kg^{-1} significantly increased feed intake (P≤0.05) without significantly affecting weight gain (Table 4), and increased tibia ash content (P≤0.05). Weight gain and toe ash content were only affected at Phyzyme XP levels of 750 FTU kg^{-1} or higher. Feed efficiency and mortality were not affected by treatment.

3.3. Trial 3

One pen trial with 1764 chickens for fattening at the age of 1 to 42 days (1 - 21 days = starter period, 21 – 42 days = finisher period) was performed. The chicks were allocated into 48 pens, in a randomised complete design, consisting of seven dietary treatments with six replicates per treatment. The basal diet contained 2.6/2.2 g kg^{-1} available P (starter/finisher diet). Phyzyme XP was tested at 0, 250, 500, 1000, 2000 FTU kg^{-1}. For comparison, a positive control containing 3.6/3.2 g kg^{-1} available P was included. At the end of the 6th week, the tibia of one bird from each pen was dissected.

Addition of Phyzyme XP at 250 FTU kg^{-1} resulted in a significant increase (P<0.05) of the final weight and feed intake of chickens (Table 5). Polynomial analysis of the phytase response revealed highly significant linear increase in feed intake and final body weight. Overall, feed efficiency was improved (P≤0.05) with 500 FTU kg^{-1} Phyzyme XP. Mortality to 42 days was not affected by treatment. Additions of phytase above 250 FTU kg^{-1} significantly increased (P≤0.05) bone ash percentage compared to the negative control diet.

Table 5. **Performance of chickens for fattening fed diets with graded levels of Phyzyme XP (1-42 days)**

Phyzyme XP (FTU kg^{-1})	Final weight (g)	Feed intake (g $bird^{-1}$)	Feed conversion (gain/feed)	Mortality (%)	Bone Ash (%)
Positive control	1982^{bc}	3544^{bc}	0.522^{a}	7.14	50.38^{de}
0	1791^{a}	3175^{a}	0.544^{ab}	3.97	44.83^{a}
250	1919^{b}	3371^{b}	0.560^{ab}	5.95	47.14^{b}
500	1982^{bc}	3424^{b}	0.573^{b}	4.76	47.43^{bc}
1000	2034^{cd}	3554^{bc}	0.565^{ab}	4.36	49.46^{cd}
2000	2109^{d}	3631^{c}	0.566^{ab}	2.78	48.43^{bcd}

$^{a, b, c, d, e:}$ Means in a column not sharing a common superscript are significantly different (P<0.05)

3.4. Trial 4

One digestibility trial with chickens for fattening from 8 to 22 days was performed. A total of 192 broilers (six chicks per pen and eight pens per treatment) were assigned to four diets in a way that the average weight of chicks was similar across dietary treatments. The basal diet contained 3.9 g kg^{-1} total P. Phyzyme XP was tested at 0, 500 and 1000 FTU kg^{-1}. For comparison, a positive control containing 7.7 g kg^{-1} total P was used. Enzyme activity in feed was measured and found appropriate.

When compared with the negative control diet, supplementation with 500 FTU Phyzyme XP kg^{-1} diet increased P retention and digestibility (54.5 *vs.* 66.4 and 53.1 *vs.* 68.8, P≤0.001, respectively). Ca retention or digestibility was not affected by the supplementation with Phyzyme XP.

3.5. Conclusions regarding efficacy

Three pen trials conducted over the 42-day growing period showed significant effects on feed efficiency, daily weight gain and/or feed intake with the supplementation of 500 FTU Phyzyme XP kg^{-1} feed. One digestibility trial demonstrated a significantly higher P retention and ileal digestibility in chickens given Phyzyme XP at 500 FTU Phyzyme XP

kg^{-1} feed. The increased bioavailability of P is demonstrated directly by increased ileal digestibility in one study, and indirectly by bone ash in three studies.

Only two studies were provided with supplementation of diets with 250 FTU Phyzyme XP kg^{-1} feed, both showing significant effects on weight gain, feed conversion and feed intake in two studies and on bone ash in one study.

The FEEDAP Panel thus concludes that the efficacy of Phyzyme XP has been demonstrated at the dose of 500 FTU kg^{-1}, but insufficient data is provided to demonstrate efficacy at the minimum recommended dose of 250 FTU kg^{-1}.

4. Safety

4.1. The safety aspects of the genetic modification

4.1.1. Information relating to the GMM and comparison of the GMM with its conventional counterpart

a) Description of the genetic trait(s) or phenotypic characteristics and any new trait which can be expressed or no longer expressed

The final production strain ATCC 5233 expresses the 6-phytase *appA* gene from *E. coli* B. It contains the expression cassette integrated into the chromosome. The final production strain does not contain any antibiotic resistance marker. This was verified by a PCR with a detection sensitivity of $\leq$ 3 pg DNA, which is sensitive enough to detect a single copy of the *bla* gene in the genome.

b) Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified micro-organism

The applicant has provided a description of all the elements in the insertion cassette. It consists of the cytomegalovirus promoter, the 5' untranslated region of human lipocortin1 cDNA, the phytase ORF, the terminator and the 3' untranslated region of the gene encoding human lipoprotein1. The applicant has provided an acceptable risk assessment of the virus sequences that are present in the final production strain.

4.1.2. Conclusions regarding the genetic modification

Phyzyme XP contains the 6-phytase enzyme produced after fermentation with the GM *Schizosaccharomyces pombe*. In the final production strain no harmful sequences have been introduced, nor are there any antibiotic resistance marker sequences.

The safety of the donor, *E. coli* B, and recipient organism *Schizosaccharomyces pombe,* is based on the concept of familiarity. For the donor organism, *E. coli* B, extra safety evidence is given by the absence of genes encoding virulence factors and the lack of pathogenic potential in both mouse and chick models.

After fermentation the enzyme product is purified so that no culturable production organisms are present and recombinant DNA is below the level of detection.

4.2. Safety for the target species

One tolerance trial with 756 chickens for fattening from 1 to 42 days (0 - 21 days = starter period, 21 – 42 days = finisher period) was performed. The chicks were allocated into 48 pens, in a randomised complete design, consisting of three dietary treatments with six replicates per treatment. The basal diet contained 2.6/2.2 g kg^{-1} available P (starter/finisher diet). Phyzyme XP was tested at 0, 500 (2X minimum recommended dose), 7500 (7.5X maximum recommended dose) FTU kg^{-1} (confirmed by recovery analysis).

No negative effects were observed with the supplementation of Phyzyme XP at 7.5 times the maximum recommended dose. In fact, feed intake and final weight were

significantly improved with the overdose of enzyme. No effects on feed conversion or mortality were observed.

Since this study was not performed at 10 times the maximum recommended dose, it does not fully satisfy the requirements for tolerance tests. Therefore, the safety for chickens for fattening has only been demonstrated at 7.5 times the maximum recommended dose.

4.3. Safety for the Consumer

4.3.1. Genotoxicity studies

The product tested in the genotoxicity assays was defined as RTA (refined test article) which was the production broth with cells removed but prior to adding stabilisers and preservatives. It was provided in lyophilised form and designated DV006R in study reports.

4.3.1.1. Bacterial Reverse Mutation Assay

The product as defined above was tested in compliance with GLP in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2*uvr*A (pKM101) both with and without microsomal enzyme activation systems. There was no evidence for genotoxicity of the test article in this study.

4.3.1.2. Mouse Lymphoma Assay

The product as defined above was tested in compliance with GLP for ability to induce forward mutations at the Thymidine kinase (TK) locus in the mouse lymphoma L5178Y cell line and was tested both with and without microsomal enzyme activation systems up to the highest non-cytotoxic dose. There was no evidence for any genotoxicity in the results of this assay.

4.3.1.3. Mammalian Micronucleus Assay

The product as defined above was tested in compliance with GLP for *in vivo* clastogenic activity by detecting micronuclei erythrocytes in mouse bone marrow following oral gavage administration of single doses of 500, 1000 and 2000 mg kg^{-1} bw. The study revealed no evidence of any toxicity to the bone marrow and showed no effect of the test article on the frequency of micronuclei.

4.3.2. Oral toxicity studies

The test article was provided in two forms for the oral toxicity studies: the Unrefined Test Article (UTA) which was the production broth with most of the cells removed and the RTA which was the same as that used for the genotoxicity assays.

4.3.2.1. 14-day range-finding study

Groups of 10 rats of each sex were administered test article daily by gavage for 14 days at doses which were multiples of the intended target species dose (50 units kg^{-1} bw day^{-1}). The administered doses were either 0, 100X, 600X, 1000X UTA or 2000X RTA. The study was conducted in compliance with GLP. Since no adverse effects were seen at the highest dose after 14 days these doses were selected for the 90-day study.

4.3.2.2. 90-day study

The dose levels used and the mode of administration were as described above for the 14-day study. Groups of 20 rats per sex per group were allocated for the low dose and intermediate group and 25 per sex per group for the control and highest dose of each test article. For each group which originally had 25 animals 20 were killed after 90 days

but the remaining five per group were kept for a further two weeks without treatment with test article before being brought to necropsy. The study was conducted in compliance with GLP and according to a current guideline protocol, and included a full range of clinical, haematological and pathological endpoints. There were no effects of treatment on clinical observations, body weight or food intake. Haematological and clinical chemistry results were similar for all groups although the females in both RTA and UTA highest dose group had globulin levels and albumin/globulin ratios which were statistically significantly higher than those of controls; a similar pattern was not seen in males. In the rats which were left for two weeks without treatment before necropsy the globulin levels of the same group were still higher than controls to a similar extent although the difference was not statistically significant, probably due to the smaller numbers of animals at this time-point.

Necropsy and histopathological examination showed no treatment-related changes apart from inflammatory changes in the lung which are identified as being consistent with the inhalation of a foreign substance. These effects are considered by the applicant and the contract laboratory to be due to the physiological effect of the high concentration of the test article leading to inhalation of particles after gavage. Although this is an unusual finding with such a product it is a known consequence of the use of gavage administration, and according to information provided by the applicant it has been seen with an enzyme product previously. It is likely that the differences seen in globulin levels are secondary to these pulmonary effects.

The FEEDAP Panel concludes that the effects seen are most likely to be a local effect of inhalation exposure, particularly to the unrefined product, and do not represent a relevant adverse effect for the purposes of safety evaluation. In the absence of any systemic effects of treatment the highest dose tested is concluded by the FEEDAP Panel to be the NOEL for this product.

4.3.3. Conclusions regarding consumer safety

On the basis of the results of three genotoxicity studies there is no concern regarding the genotoxicity of this product.

Although effects were seen in the 90-day study with this product they are considered by the FEEDAP Panel to be artefacts of the testing procedure used and as such not relevant to the safety evaluation. The FEEDAP Panel concludes that there is no evidence for concern regarding consumer safety.

4.4. Safety to the User

4.4.1. Skin irritation

When 0.5 mL of the UTA product was applied to the skin of three male and three female rabbits and left under semi-occluded dressing for four hours there was a slight erythema in five of the animals, which remained in one case for 24 hours. This is interpreted as evidence of slight irritancy. The study was conducted in compliance with GLP.

4.4.2. Eye irritation

When the UTA product was applied directly to the eye of three male and three female rabbits and apart from a slight redness in some animals immediately after treatment no irritant effects were observed. The study was conducted in compliance with GLP. The product is concluded to be non-irritant to the eye from the results obtained.

4.4.3. Sensitisation

A study was conducted in guinea pigs to the Buehler protocol and according to GLP. Groups of ten guinea pigs of each sex were tested against a control group of five of each

sex using the UTA product. There was no evidence of delayed contact hypersensitivity in this test.

4.4.4. Acute inhalation toxicity

Five male and five female rats were exposed to an aerosol of 2.2 mg L^{-1} of the UTA product for six hours and observed for 14 days. The study was conducted in compliance with GLP. A gross necropsy but no histological examination was performed. Dark material was reported around the nose and mouth of the treated group on the day of treatment but no mortality or other adverse effects were reported.

4.4.5. Conclusions regarding user safety

The product, even in its most unrefined form, shows little evidence of irritancy, low acute inhalation toxicity and shows no signs of dermal sensitisation potential. Although the pulmonary effects seen in the 90-day study are considered an artefact of the testing procedure they may indicate some inherent potential for the product to cause inflammation if inhaled. By convention the product is labelled as a potential sensitizer and thus protective clothing is recommended.

4.5. Environmental Safety

4.5.1. Potential environmental impact of the genetic modification

No environmental impact from the use of this product is expected on the basis of the sequences incorporated and the characteristics of the recipient micro-organism. The production micro-organism is removed from the product and the recombinant DNA is below the limit of detection.

4.5.2. Environmental impact of feed additive

The active ingredient is a 6-phytase enzyme which is a natural substance and is considered of no environmental concern. Thus, no further environmental assessment is required.

CONCLUSIONS

On the basis of the data submitted, the GMO Panel concluded that it is unlikely that the genetic modification of *S. pombe* to produce Phyzyme XP has any adverse effects on human and animal health or the environment.

Three pen trials conducted over the 42-day growing period showed significant effects on feed efficiency, daily weight gain and/or feed intake with the supplementation of 500 FTU Phyzyme XP kg^{-1} feed. P digestibility was also demonstrated to be increased with 500 FTU Phyzyme XP kg^{-1} feed. Only two studies were provided with supplementation of diets with 250 FTU Phyzyme XP kg^{-1} feed, both showing significant effects on weight gain, feed conversion and feed intake in two studies and on bone ash in one study. The FEEDAP Panel thus concludes that the efficacy of Phyzyme XP has been demonstrated at the dose of 500 FTU kg^{-1}, but insufficient data is provided to demonstrate efficacy at the minimum recommended dose of 250 FTU kg^{-1}.

A tolerance test carried out with chickens for fattening over a six-week period with a 2X minimum recommended dose (500 FTU kg^{-1}) and 7.5X maximum recommended dose (7500 FTU kg^{-1}) of Phyzyme XP produced no evidence of any adverse effect on performance or mortality. The FEEDAP Panel therefore concludes that the safety of Phyzyme XP has been demonstrated at 7.5X the maximum recommended dose (7500 FTU kg^{-1}). However, the tolerance of this product has not been demonstrated at 10X the maximum recommended dose.

On the basis of the results of three genotoxicity studies there is no concern regarding the genotoxicity of this product.

Although effects were seen in the 90-day study with this product they are considered by the FEEDAP Panel to be artefacts of the testing procedure used and as such not relevant to the safety evaluation. The FEEDAP Panel concludes that there is no evidence for concern regarding consumer safety.

The product, even in its most unrefined form, shows little evidence of irritancy, low acute inhalation toxicity and shows no signs of dermal sensitisation potential. Although the pulmonary effects seen in the 90-day study are considered an artefact of the testing procedure they may indicate some inherent potential for the product to cause inflammation if inhaled. By convention the product is labelled as a potential sensitizer and thus protective clothing for this purpose should be sufficient to minimise inhalation exposure, assuming that the clothing includes respiratory protection.

The FEEDAP Panel concludes that there is no evidence for concern regarding safety for the environment.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier Phyzyme™ XP Chickens for fattening. December 2002. Submitted by Danisco Animal Nutrition.
2. Answers to Member State questions. October 2005. Submitted by Danisco Animal Nutrition.
3. Answers to EFSA questions. November 2005. Submitted by Danisco Animal Nutrition.
4. Answers to EFSA questions – Supplementary information. January 2006. Submitted by Danisco Animal Nutrition.
5. Supplementary information. March 2006. Submitted by Danisco Animal Nutrition.

REFERENCES

1. IFBC, International Food Biotechnology Council. 1990. Biotechnologies and Food: Assuring the Safety of Foods produced by Genetic Modification. National Academic Press, Washington DC. pp. S114-S128.
2. OECD. 1992. Safety Consideration for Biotechnology. Organization for Economic Cooperation and Development. OECD Council.

FEEDAP PANEL MEMBERS

Arturo Anadón, Margarita Arboix Arzo, Georges Bories, Paul Brantom, Joaquim Brufau de Barberà, Andrew Chesson, Pier Sandro Cocconcelli, Joop de Knecht, Noël Dierick, Gerhard Flachowsky, Anders Franklin, Jürgen Gropp, Ingrid Halle, Anne-Katrine Lundebye Haldorsen, Alberto Mantovani, Kimmo Peltonen, Guido Rychen, Pascal Sanders, Amadeu Soares, Pieter Wester and Wilhelm Windisch

GMO PANEL MEMBERS

Hans Christer Andersson, Detlef Bartsch, Hans-Joerg Buhk, Howard Davies, Marc De Loose, Michael Gasson, Niels Hendriksen, John Heritage, Sirpa Kärenlampi, Ilona Kryspin-Sørensen, Harry Kuiper, Marco Nuti, Fergal O'Gara, Pere Puigdomenech, George Sakellaris, Joachim Schiemann, Willem Seinen, Angela Sessitsch, Jeremy Sweet, Jan Dirk van Elsas and Jean-Michel Wal

ACKNOWLEDGEMENT

The GMO Panel wishes to thank Lieve Herman, Bevan Moseley and Ingolf Nes for their contributions to this opinion.