EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B3 - Management of scientific committees II

Opinion of the SCAN on the use of enzymatic product Natuphos® 5000 (3-phytase; EC 3.1.3.8) as feed additive (adopted on 27 April 2000)

1. TERMS OF REFERENCE

The Scientific Committee on Animal Nutrition is requested to evaluate the dossier submitted for the permanent authorisation of product Natuphos 5000 ®. In its assessment the Committee is requested to assess both the efficacy at the claimed level(s) of use and the safety of the product.

2. BACKGROUND

The additive Natuphos ® 5000 is a dry product consisting of the enzyme phytase mixed with wheat middlings as a carrier. Natuphos ® 5000 is produced in cooperation by Gist–Brocades (The Netherlands) and BASF AG (Germany). The active substance phytase is produced via fermentation by Gist-Brocades and the commercial product Natuphos ® 5000 is formulated by BASF.

Product 3-phytase (Natuphos® 5000) (EC 3.1.3.8) as produced by *Aspergillus niger* strain FTU-8 (CBS 114.94) was given a provisional Community authorisation in April 1994. Presently the company (BASF & Gist-Brocades) requests a permanent authorisation of its product.

Natuphos®5000 is intended to be used in piglets, pigs for fattening and sows at a recommended dose of 400-500 FTU/kg[1] complete feedingstuff (equivalent to 80 - 100 g Natuphos per ton of feed), particularly in diets with a minimum content of 0.23% phytin bound phosphorus (e.g. 20% wheat diet, tapioca, oilseeds and pulses). The company identified a minimum effective dose of 125, 200 and 500 FTU for piglets, pigs for fattening and sows respectively.

It is also proposed for chickens for fattening 500-700 FTU/kg (equivalent to 100 – 140 g Natuphos per ton of feed) and laying hens 300 - 400 FTU/kg (equivalent to 60 – 80 g Natuphos per ton of feed) in diets with a minimum content of 0.3 % phytin bound phosphorus . In addition the company identified a minimum dose of 125 and 100 FTU for chickens for fattening and laying hens respectively.

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[1] FTU: one unit FTU of phytase is defined as the quantity of enzyme which liberates 1µmol of inorganic phosphate per minute from sodium phytate at pH 5.5 and 37 ºC

N°.	Additive	Chemical formula, description	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	Period of authorisation
					Units of activity per kg of complete feedingstuff			
1	3-phytase EC 3.1.3.8 **[Natuphos]**	Preparation of 3-phytase produced by *Aspergillus niger* (CBS 114.94) having a minimum activity of: Solid and liquid forms: 5 000 FTU/g [(2)]	Piglets	**4 months**	**125 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 kilogram of complete feedingstuff: **400-500 FTU** 3. For use in compound feed containing more than 0.23 % phytin bound phosphorous	Without a time limit
1			Pigs for fattening	-	**200 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 kilogram of complete feedingstuff: **400-500 FTU.** 3. For use in compound feed containing more than 0.28 % phytin bound phosphorous	Without a time limit
1			Sows	-	**500 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 kilogram of complete feedingstuff: **500 FTU** 3. For use in compound feed containing more than 0.36 % phytin bound phosphorous	Without a time limit

[2] One FTU is the amount of enzyme which liberates one micromole of inorganic phosphate per minute from sodium phytate at pH 5,5 and 37°C

N°	Additive	Chemical formula, description	Species or category of animal	Maximum age	Minimum content	Maximum Content	Other provisions	Period of authorisation
					Units of activity per kg of complete feedingstuff			
1			Chickens for fattening	-	**125 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 kilogram of complete feedingstuff**: 500-700 FTU** 3. For use in compound feed containing more than 0.36 % phytin bound phosphorous	Without a time limit
1			Layers	-	**100 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 kilogram of complete feedingstuff**: 300-400 FTU** 3. For use in compound feed containing more than 0.36 % phytin bound phosphorous	Without a time limit

3. CHARACTERISATION OF THE ADDITIVE

The dossier provides a detailed description of Natuphos®5000. The enzyme concentrate has a phytase activity of at least 5000 FTU/g additive and represents some 10-20 % w/w of the final product, with the bulk being made up with wheat-middlings. The carrier forms between 80-90% w/w.

Optimal pH lies between 2 and 6, with a maximal activity at pH 5.5. Optimal temperature is 55ºC. The product is stable in an aqueous environment (100% stable for 6 hours at room temperature). At 4ºC, it is stable for at least 12 months. Loss of phytase activity after storage for 6 months at 21ºC was low. At 40ºC and 70% relative humidity, the activity fell to 22% of that originally present.

Pelleting at 70ºC resulted in 20% loss of activity. However, at 80ºC the losses were considerably higher (80%). Stability was also studied in premixes (losses of 40%) and feed (losses of 20-50%) after storage of feed for 8 weeks at 21ºC and 30ºC respectively). Generally, moisture and high temperature have a negative effect on the stability of phytase.

Recently the company presented the product in a new formulation Natuphos 5000 G comprising of 12 % enzyme, 82 % starch and 6 % $MgSO_4$.The new formulation is intended to improve product stability during storage and the pelleting of the final feed. The nutritional bioequivalence of this product in relation with the powder and liquid has been determined studying the live weight gain and the ash percentage on the toe of broilers of 14 days.

Incompatibilities: The stability of phytase can be adversely affected by water or additives containing water (e.g. salts).

4. EFFICACY

4.1. General scientific knowledge

The objective of phytase supplementation of diets of non-ruminants is to increase the digestibility of phosphorus in feeds and to reduce the phosphorus excretion by animals. Some 50 to 75% of phosphorus present in plant feedingstuffs is bound in the form of phytin.

Data on the efficacy of 3-phytase in diets for pigs and poultry is published in many scientific journals, involving measurements of performance, apparent absorption and retention of phosphorus and bone characteristics. Reviews on the effect of phytases (Campbell and Bedford 1992; Kornegay 1996; Kemme and van der Klis 1997) clearly confirm that microbial phytase is very effective for improving the availability of phytate-phosphorus, especially in maize and soya bean diets.

It has also been demonstrated that the magnitude of the response to added phytase is inversely related to the level of available phosphorus and the level or amount of supplementary phytase added. Phytase supplementation in pigs and poultry makes it possible to meet phosphorus requirements with much lower levels of added phosphorus in the diet by increasing the availability of

the phytin phosphorus from vegetable feedingstuffs. This results in a reduction of up to 30-50% in the phosphate excretion into the environment.

For poultry, body weight gain, apparent retention and excretion of phosphorus, and percentage of ash in tibia, are the most sensitive measurements, followed by feed intake and resistance to shear force of tibia. Apparent retention of calcium, nitrogen and dry matter and shear stress of tibia are the least sensitive indicators(Kornegay 1996).

For pigs, the apparent absorption of phosphorus and percentage of ash of the tenth rib and metacarpus are the most sensitive measurements followed by body weight gain, feed intake and phosphorus excretion (Kornegay 1996).

4.2. Studies considered in the dossier (Table 1)

Category of target animal	**Type of studies**	**Reference**
Growing pigs	Balance	Beers 1992
Piglets	Balance	Lantzsch *et al* 1992
Piglets, growing pigs	Balance	Pallauf *et al* 1992
Piglets	Balance	Jongbloed *et al* 1990
Growing pigs	Balance	Jongbloed *et al* 1992.
Piglets	Balance	Hoppe *et al* 1992
Piglets, growing pigs, sows, midpregnancy sows, lactating sows	Balance	Kemme *et al* 1997
Growing pigs, Broilers	Balance Animal performance	Simons *et al* 1990
Broilers	Balance	Schöner *et al* 1991
Laying hens	Balance	Van der Klis *et al* 1991
Laying hens	Animal performance	Simons *et al* 1992
Laying hens	Balance Animal performance	Van der Klis *et al* 1997

The minimum doses identified by SCAN are based on data presented by the company. As a principle, SCAN considered the lowest efficacious dose-level of Natuphos 5000 was given when at least three significant improvement of phosphorus digestibility ($P<0.05$) were provided in three different experiments.[3]

[3] Minority opinion: Professor Flachowsky abstained on the way to confirm the lowest effective dose levels as he considered that the general rules on how to assess the efficacy of enzymatic products used as feed additives (at least three significant results) were adopted by SCAN recently and the company did not know these rules during preparation of the dossier.

For approval of lower doses than those identified by SCAN as efficacious, additional studies should be conducted.

4.3. Efficacy in pigs

Two sets of pig data were available in the dossier. See Table 2 hereafter.

The first set of studies were dated 1990 –1992 (Jongbloed and Kemme 1990, Beers. 1992, Hoppe et al 1992, Jongbloed et al 1992, Lantzsch & Wjst 1992, Pallauf et al 1992 and Simons et al 1990). These involved nine phosphorus-digestibility trials, with and without phytase, and demonstrated that the addition of phytase increased significantly ($P<0.05$) the digestibility of phosphorus in all experiments and reduced the amount of phosphate in the manure.

Beers (1992), reported in two trials using 256 pigs (starter live weight 25 kg) the phosphorus digestibility of two different diets (A: maize/soybean, B: more by-products) to which were added six increasing levels of phytase Natuphos from 200 - 1780 FTU/kg. Improvements were significant ($P<0.05$) with both diets, but better for the maize/soybean diet.

In another, more recent, set of experiments made with 112 piglets, 32 growing-finishing pigs and 12 sows (Kemme *et al*., 1997). All animals were fed with identical feedingstuffs with or without 500 FTU phytase/kg. Addition of phytase FTU-8 to diets of all categories of pigs showed significant positive effects ($P<0.05$) on phosphorus digestibility and, in addition, lowered phosphorus excretion and thus reduced environmental pollution.

From these data it can be seen that the digestibility of phosphorus increased to 8.3 percentage units as the body weight of pigs increased from 30 to 60 kg and then remained stable until 100 kg.

Pregnant sows had a lower efficiency of phosphorus absorption than piglets and growing-finishing pigs. During lactation, the efficiency of phosphorus absorption was 3.4 percentage units higher than during pregnancy but was still 6.6 % lower than for growing pigs. The efficacy of phytase in generating digestible phosphorus decreased in the order of lactating sows, growing pigs, sows at the end of pregnancy, piglets and sows at mid pregnancy. From this it can be concluded that the amount of phytase added to diets should vary according to the physiological status of a target category (Kemme et al 1997).

As it can be seen in the Table 2, Natuphos® enzyme preparation improved the digestibility of phosphorus by 20-30% and its use markedly decreased the excretion of phosphorus in the excreta. From piglets to sows, differences in the phosphorus digestibility in the control and experimental groups have been shown significant at least at the $P<0.05$ level.

Table 2. Phosphate digestibility experiments in pigs with or without phytase

It should be noted that the company provided a separate document attesting that all sources of phytase used originated from *Aspergillus niger* strain FTU-8 (CBS 114.94). In some cases, the sources of phytase are identified as *Aspergillus ficuum*, which is synonymous with *Aspergillus niger*.

No. of animals	Weight of animals (kg)	Duration of experiment	Comp. of diet	Conc. Of phytase (FTU/kg)	Phosphorus Digestibility (%) Experimental group	Phosphorus. Digestibility (%) Control group	Product involved	Reference
Piglets : weight of animals lower than 25kg during most of the experimental period								
30	10-25 Piglets	3 weeks	Barley, soybean	1000	72*	45	Natuphos ®	Lantzsch & Wjst 1992
16	10-15 Piglets	1 week	Maize, soybean	500 1000	54* 64*	28	Natuphos ®, Gist Brocades, *Aspergillus niger* (*A. ficuum*)	Pallauf et al 1992
16	20-25 Piglets	1 week	Maize, soybean	500 1000	57* 64*	30	Natuphos ® *Aspergillus niger*, Basf	Pallauf et al 1992
24	7-32 Piglets	6 weeks	Maize soybean	125 250 500 1000	43* 41* 55* 63*	30	Natuphos ® *Aspergillus niger*	Hoppe et al 1992
Pigs for fattening : weight of animal above 25 kg in the major part of experimental period								
112	30-40	3 weeks	Standard diet	500	37*	23	Natuphos ® *Aspergillus niger*	Kemme et al 1997
128	25-49	5 weeks	Maize soybean	220 440 720 920 960 1780	47* 52* 57* 62* 59* 61*	29	*Aspergillus niger* Gist brocades	Beers, 1992
128	25-49	5 weeks	Maize soybean byproducts	280 480 660 880 930 1730	38* 47* 53* 57* 57* 61*	30	*Aspergillus niger* Gist Brocades	Beers, 1992
36	25-50	5 weeks	Maize , soybean, byproducts	200 400 700 1000 2000	42* 48* 55* 60* 60*	30	*Aspergillus niger* (var. *ficuum*) NRRL 3135 Gist Brocades	Jongbloed et al 1990
6	37-80	8 week	Maize – soybean, standard diet	1500 1500	42* 54*	12 27	*Aspergillus niger* (var. *ficuum*) NRRL 3135 Gist Brocades	Jongbloed et al 1992
22	35-70	5 weeks	Maize soybean Practical diet	1000 1000	46* 56*	20 34	*Aspergillus ficuum* NRRL 3135	Simons et al 1990
32	40-100	10 weeks	Standard diet	500	43*	26	Natuphos ® *Aspergillus niger*	Kemme et al 1997

Table 2 (continued)

No. of animals	Weight of animals (kg)	Duration of experiment	Comp. of diet	Conc. Of phytase (FTU/kg)	Phosphorus Digestibility (%) Experimental group	Phosphorus. Digestibility (%) Control group	Product involved #	Reference
Reproductive Sows: pregnancy and lactation								
12	Sows Lactation (4 studies)	60 d of Pregnancy	Standard diet	500	20 *	13	Natuphos ® *Aspergillus niger*	Kemme et al 1997
		100 d of Pregnancy			33*	18		
		10 d of lactation			40*	19		
		25 d of lactation			41*	19		

- * : = P < 0.05
- Standard diet e.g. (Kemme et al 1997 : 20 % wheat middlings, 31% corn, 20% tapioca, 7.5% peas, 5% potato protein, 5% soybean, 5 % sunflower, 3 % cane molasses, 1.6% rendered fat, 1.1% limestone , 0.1% cornstarch + mix of trace and vitamins) ,
- Practical diet e.g. (Simons et al . 1990 : 12% soybean, 42.1 % tapioca, 33% hominy feed USA, 8% sunflower, 2.6 % soya-bean oil, 0.8% limestone, + mix of trace and vitamins)

Conclusion:

From the available results, having taken into account all dose-levels studied and their reproducibility, it can be concluded that the minimal efficacy dose is demonstrated

- In the case of piglets, at the dose of at least 500 FTU/kg feed (maize-soya or standard diets),
- In the case of pigs for fattening, at the level of 280 FTU/kg and
- In the case of sows at the level of 500 FTU/kg

Therefore, the efficacy of *Aspergillus niger* phytase Natuphos 5000 when used as an additive in dry pig feeds for the increase of phosphorus digestibility is demonstrated at the recommended dose range of 400 - 500 FTU/kg of feed. But as far as the minimum effective content proposed by the company respectively for piglets at 125 FTU and for pigs for fattening at 200 FTU are concerned, they still need confirmation with further studies.

4.4. Efficacy in poultry

4.4.1. Data available

Table 3 : Phosphate digestibility experiments in broilers and laying hens with and without phytase

It should be noted that the company provided a separate document attesting that all sources of phytase used originated from *Aspergillus niger* strain FTU-8 (CBS 114.94). In some cases, the sources of phytase are identified as *Aspergillus ficuum*, which is synonymous with *Aspergillus niger*.

Number of animals	Duration of experiment	Composition. of the diet	Concentration of phytase (FTU/kg)	Phosphorus. Digestibility Experimental Group (%)	Phosphorus Digestibility Control Group (%)	Product Involved	Reference
Chickens for fattening (broilers)							
176 Broilers	0-24 days 21-24 days P. Availability Test	Standard diet	250 500 750 1000 1500	56 * 59* 59 * 62 * 64 *	49	Aspergillus ficuum NRRL 3135	Simons et al 1990
168 Broilers	0-28 days 21-24 days P. Availability Test	Standard diet	375 750 1500 2000	60 * 62 * 62 * 62 *	51	Aspergillus ficuum NRRL 3135	Simons et al 1990
2880 Broilers	0-42 days 21-24 day P. Availability test	Maize soybean	125 250 500 1500	52 * 54 * 58 * 64 *	48	Aspergillus niger, Basf	Schöner et al 1991
Layers							
768 laying hens	Week 20 to 24	Maize soybean	250 500 250 500	53 * 58 * 58 * Phy d. ¤ 70 * Phy d. ¤	28 37 Phy d. ¤	Gist brocades	Van der Klis et al 1991
768 laying hens	Week 21 to 22	Maize soybean	250 500 250 500	49 * 52 * 59 * Phy d. ¤ 71 * Phy d. ¤	22 21 Phy d. ¤	Natuphos®, Aspergillus niger,Gist brocades.	Van der Klis et al 1997
768 laying hens	Week 22 to 35	Maize soybean	250 500	94 % egg p.* 93 % egg p.*	89 % egg p.	Natuphos®, Aspergillus niger,Gist brocades.	Van der Klis et al 1997
512 laying hens	Week 18 to 68	Maize soybean	100 200 300	90 % egg p.* 88 % egg p.* 89 % egg p.*	84 % egg p.	Natuphos®, Aspergillus niger,Gist brocades.	Van der Klis et al 1997
288 laying hens	Week 24 to 52	Maize soybean	200 300 450 670 1000 1500 2000	96% egg p.* 96* 95* 95* 96* 96* 97*	78 % egg p.	Aspergillus Niger, Gist brocades	Simons et al 1992 . Spelderholt No 568

*: P<0.05 ; ¤ : % Phy.d : percentage of phytin degradation at intestinal level. ; egg p. : production of egg expressed in percentage

Standard diet for broilers : Simons et al 1990 : 28% maize, 20% sorghum, 8% sunflower, 35 % soybean, 6% soya-bean oil, 1% limestone + mix of mineral and vitamins.

4.4.2. Broilers

The efficacy of Natuphos on phosphorus availability of feeds in broilers was examined in three studies (Simons *et al.*, 1990 and Schöner *et al.*, 1991). The efficacy of phytase was investigated using a dose-response study. Growth and feed conversion ratios were measured during the experimental periods. Apparent availability of total phosphorus was measured over a 3-day period, during which feed consumption was measured as dry matter intake and excreta were collected quantitatively (balance study).

In the first experiment 250, 500, 750, 1000 and 1500 units phytase/kg were added to the basal diet. The second experiment was intended to examine responses to a higher dose range and used 355, 750, 1500, 2000 units phytase/kg.

In the third experiment a dose response (125, 250, 500, 1500 units phytase/kg) was examined in relation to four levels of inorganic phosphorus and three levels of calcium.

Phytase supplementation increased significantly ($P<0.05$) the digestibility of phosphorus in these experiments and reduced the phosphorus in manure.

As it can be seen in the Table 3, Natuphos® enzyme preparation improved the digestibility of phosphorus by 8-30% and its use markedly decreased the excretion of phosphorus in the excreta of broilers. The differences on the phosphorus digestibility from control diet and all experimental groups have been shown to be significant at least at the $P< 0.05$ level.

Conclusion

From the available results, having taken into account all dose-levels studied and their reproducibility, it can be concluded that the lowest efficacious dose-level in chickens for fattening (broilers) is demonstrated at the level of 375 FTU/ kg

The minimum content of Natuphos for chickens (125 FTU/kg) proposed by the company cannot be recommended as it is based on data from a single experiment.

4.4.3. Laying hens

The efficacy of Natuphos in laying hens has been determined in three sets of studies (Van der Klis *et al*., 1991, Simons *et al.*, 1993 and Van der Klis et al, 1997).

All of these experiments were intended to study the efficacy of microbial phytase application using dose responses. The effect of phytase addition was studied taking into account interactions between calcium and inorganic phosphorus in order to identify the best level of application. Results were evaluated by measuring the absorption of Ca, P, N, organic matter and phosphorus phytate (balance nutritional study). The performance of the laying hens and egg quality were also measured. The efficacy of phytase was statistically significant at $P< 0.05$. Phytase at an application rate of 250 and

500 FTU/kg of feed increased phosphorus digestibility by 40 and 50 % respectively.

Conclusion

The lowest effective dose–level of the product is demonstrated at the level of 250 FTU/kg. The lowest level proposed by the company for laying hens (100 FTU/kg) cannot be supported because the data were based on a single experiment. However the recommended use of 250-400 FTU for laying hens is well supported by the experimental data.

5. SAFETY

The enzyme phytase is produced from a genetically modified *Aspergillus niger* GAM-53 in facilities controlled by local existing legislation for handling genetically modified micro-organisms on Good Industrial Large Scale Practice (GILSP). The host strain *A. niger* GAM-53 was derived from the parental strain *Aspergillus niger* (V. Tieghem, *ficuum*) NRRL 3122. It is a non-pathogenic selected mutant. It has had a long history of use in producing enzymes (e.g. amyloglucosidase) for the food industry. The host organism also appears in the literature under the synonyms *Aspergillus niger* (var *ficuum*) or *Aspergillus ficuum* (Hennings 1895).

This strain was genetically modified after transformation by the plasmid pFYT 3 harbouring the 3-phytase gene to obtain the strain FTU-8 (CBS 114-94). The gene coding for the phytase enzyme was isolated from *A. niger* NRRL 3135 and combined with the homologous amyloglucosidase promotor sequence and the end S marker sequence, both derived from *Aspergillus niger*, associated for selection with a gene from *A. nidulans* coding for an acetamidase. Multiple copies of this construct were introduced into the host strain *Aspergillus niger* GAM-53 (CBS 513-88) to obtain the strain FTU-8 (CBS 114-94).

The enzyme is a 3-phytase (EC. 3.1.3.8) with the systematic name (IUB): myo-inositol-hexakisphosphate-3-phosphohydrolase. The enzyme preparation meets the purity criteria set for enzymes formulated by the WHO/FAO/JECFA and Food Chemical Codex (FCC). The absence of specified heavy metals, mycotoxins and antibiotics were verified, as well as an absence of contaminating micro-organisms in the production strain. Results of studies for acute oral toxicity, acute dermal irritation, acute eye irritation, subchronic toxicity, genotoxicity assays and tolerance tests on target animals, are summarised below, demonstrated the product to be safe. Concerning workers' safety, the product has to be labelled as R42 (may cause sensitisation by inhalation).

Oral toxicity: It was conducted in rats and established an oral LD50 value in excess of 5000 mg/kg bodyweight.

Acute dermal irritation: It was conducted in rabbits for 4 hours. Signs of erythema were observed during the first 24 hours of observation. The product was considered as non-irritant to skin.

Acute eye irritation: It was conducted in the rabbit and the product was found as non-irritant to eye.

Sensitising properties: A sensitisation test was not carried out but the substance is labelled as R42 meaning that the product "may cause sensitisation by inhalation".

Range-finding oral toxicity study: The product was examined in a 14-day study with groups of 5 male & 5 female rats, receiving levels of Natuphos of 0, 1250, 5000 or 20000 mg/kg diet. At the highest levels there were no signs of toxicity (equivalent to an intake of 2000 mg Natuphos/kg bodyweight/day).

Subchronic toxicity: Groups of 10 male & 10 female rats were tested for 90 days at dose levels of 0, 2500, 7500 and 22500 mg/kg diet. As no signs of toxicity were recorded, a NOEL of 22500 ppm was established (equivalent to an overall intake of c. 1.4 g phytase/kg bodyweight/day).

Genotoxicity assays: The final product was tested for the capacity to induce gene mutations using the *Salmonella*/microsome assay using strains TA1535 TA1537 TA98 and TA100; there was no evidence of mutagenicity.

The enzyme concentrate derived from strain FTU-8, that was used in Natuphos 5000, was also tested *in vivo* for genotoxicity, using the mouse micronucleus assay. Mice were given single oral doses of 0, 625, 1250 or 2500 mg/kg bw. The treatment did not cause any increase in the number of micronucleated polychromatic erythrocytes, indicating an absence of genotoxic effect in this study. As there was also no indication of mutagenic potential from the results of the bacterial assay, it was concluded that the material under test was non-genotoxic.

Tolerance test: BASF and Gist-Brocades presented supplementary data resulting from tolerance studies with broilers and piglets using phytase from FTU-8.

In both cases the tolerance test was done to verify experimentally that ten-fold overdosing of the highest recommendation level had no negative influence on performance, mortality or daily health status. The tested dosages were 1000 and 10,000 FTU/g feed compared with no enzyme addition.

In the case of broilers, the experiment lasted 22 days. Each experimental group (treatments) consisted of 80 birds, subdivided into eight replicates of 10 birds each. The birds were fed with a corn-soya diet. The health status was controlled every day. No differences were found between treatments. In the first days of life an *E.coli* infection was diagnosed but no other reason for mortality was detected. After 10 days no further birds died and the general health status of those remaining was good.

In conclusion, no negative dose-related effects were shown either on performance or mortality using Natuphos (FTU-8) at a ten-fold overdose in broiler feeds.

In the case of piglets, the experiment lasted 21 days. Each experimental group consisted of 16 piglets per treatment. Piglets were individually located and fed with cold-pelleted diets (without steam, temperature after pelleting 65ºC). Water and feed were *ad libitum*. Health status was individually examined every day. No differences were found between treatments. The highest dose of Natuphos tended to improve the feed efficiency in comparison to the other treatments. The daily health status was generally very good. In conclusion, no negative effect was found as a consequence of feeding ten-fold the recommended dose of Natuphos (FTU-8) to pigs.

A number of other issues were considered, including:

Production of secondary metabolites: The parent strain *Aspergillus niger* GAM-53 produces the toxins naptho-γ-pyrones and malformin C as secondary metabolites. The modified production strain FTU-8 has decreased capacity to produce naptho-γ-pyrones and and has lost its capacity to produce malformin C. Routine monitoring of the product has not detected these secondary metabolites. Furthermore, a series of other known fungal metabolites were tested for, but were not detected in the final preparation.

Activation of antibiotic prodution genes: Although this was not studied at the molecular level, there is a routine control of antibiotic activity, which was negative in the final product.

6. GENERAL CONCLUSION

The efficacy of phytases in general is well documented in the literature.

The present dossier was subsequently supplemented with comprehensive and sufficient data to conclude that Natuphos FTU-8 (CBS 114.94) is effective in improving phosphorus-digestibility and reducing phosphorus excretion into the environment. The lowest efficacious dose-level of Natuphos ® 5000 has been well confirmed when supplied at:

- 375 FTU/kg for chickens,
- 250 FTU/kg for laying hens,
- 280 FTU/kg for pigs for fattening and
- 500 FTU/kg for piglets and pregnant and lactating sows .

The SCAN considers that the efficacy at the minimum doses proposed by the Company for piglets (125 FTU/kg), pigs for fattening (200 FTU/ kg), chickens (125 FTU/kg) and laying hens (100 FTU/kg) has not been adequately demonstrated. More supportive data are needed before these lower application levels could be accepted.

The dossier relating to the safety aspects provided by the Company has demonstrated the safety of 3-phytase Natuphos®5000 derived from *Aspergillus niger* strain FTU-8 (CBS 114.94) when used at the above mentioned levels as feed additive for animal feeding. It has to be noticed that Natuphos 5000 is labelled as R42 as a substance which may cause sensitisation by inhalation.

References

Beers, S. (1992). Relation between the dose of microbial phytase and phosphorus digestibility in diets for pigs. Report IVVO-DLO **228**.

Campbell, G.L.; Bedford,M.R. (1992). Enzyme application for monogastric feeds: a review. Can. J. Anim. Sci. **72**: 449-466.

Hennings, P. (1895). Ustilago ficuum reich. Sterigmatocystis Ficuum. Hedwigia **34**: 86.

Hoppe, P.P.; Schöner, F.J; Wiesche, H.; Schwarz, G. (1992). Vergleich von mikrobillier Phytase und anorganischem Phosphat bei Ferkeln: Effekte auf die Leistungen, die Mineralstoff-Retention und den Minerlstoffgehalt der Phalanx I. 46th meeting of the " Gesellschaft für Ernährungsphysiologie", Göttingen.

Jongbloed, A.W.; Beers,.S.; Kemme, P.A.; Mroz, Z. (1990). Microbial phytase in pig nutrition. Lelystad, Institut voor Veevoedingdonderzoek.(I.V.V.O.; Institute for research on Livestock Nutrition),P.O. Box 160, 8200 Lelystad.

Jongbloed, A.W.; Mroz.Z.; Kemme, P.A. (1992.). The effect of supplementary *Aspergillus niger* phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of alimentary tract. J. Anim. Sci. **70**: 1159-1168.

Kemme, P.A.; Jongbloed,A.W.; Morz, Z.; Beynen, A.C. (1997). The efficacy of *Aspergillus niger* phytase in rendering phytate phosphorus available for absorption in pigs is influenced by pig physiological status. J. Anim. Sci. **75**: 2129-2138.

Kemme, P. A.; Jongbloed,A.W.; Van der Klis, J.D. (1997). The impact of microbial phytase on the nutrition of monogastrics and the environment. Feed Manufacturing in Southern Europe:New Challenges, Reus, CIHEAM.Pag : 135-145

Kornegay, E. T. (1996). Nutritional , Environmental, and Economic Considerations for Using Phytase in Pig and Poultry Diets. Nutrient Management of Food Animals to Enhance and Protect the Enviroment. E. T. Kornegay, CRC Press, Inc.

Lantzsch, H.J.; Wjst, S. (1992). Wirkung mikrobieller Phytase (*A. niger*) auf den P, Ca, Mg and Zn Stoffwechsel junger Schweine unter dem Einfluss steigender Ca-Gehalte im Futter. 46th meeting of the "Gesellschaft für Ernährungsphysiologie", Göttingen.

Pallauf, J.H.,D.; Rimbach, G.; Neusser, H. (1992). Einfluss einer Zulage an mikrobieller Phytase zu einer Mais-Soja-Diät auf die scheinbare Absorption von Phosphor und Calcium beim-Ferkel. J. Anim. Physiol. a. Anim. **67**: 30-40.

Schöner, F.J.; Hoppe, P.P.; Schwarz, G. (1991). Mastkükenversuch mit mikrobieller Phytase: Wirkungsvergleich mit anorganischem Phosphat bei variierter Calcium - Versorgung. 3 Symposium, Vitamine und weitere Zuzatzstoffe bei Mensch und Tier. 26/27.09.1991, Stadt bei Jena, 353-358

Simons, P.C.M.V.; H.A.J.Versteegh ; Jongbloed, A.W.; Kemme, P.A.; Slump. P.; Bos, K.D.; Wolters, M.G.E.; Beudeker, R.F.; Verschoor, G.J. (1990). Improvement of phosphorus availability by microbial phytase in broilers and pigs. Brit. J. of Nutr. **64**: 525-540.

Simons, P.C.M.V.; H.A.J.Versteegh (1992). The effect of microbial phytase addition to laying hen diest on hen performance and bone and egg (shell) quality. Spelderholt, Centrum voor onderzoek en voorlichting voor de pluimveehouderij; uitgave No.568

Van der Klis, J.D.V.; H,A.J.Versteegh (1991). De ileale absorptie van fosfor in lichte witte leghennen bij toepassing van micribieel futase en verschillende calcium gehaltes in leghennenvoer. Spelderholt, Centrum voor onderzoek en voorlichting voor de pluimveehouderij.

Van der Klis, J.D.V., H.A.J.Versteegh ; Simons, P.C.M.; Kies, A.K. (1997). The efficacy of phytase in corn-soybean meal-based for laying hens. Poultry Sci **76**: 1535-1542.