

## Short communication

# IgE-mediated allergy to phytase – a new animal feed additive

**Background:** Although fungal phytase is frequently used as an additive to animal feed few investigations of its allergenic property have been conducted.

**Methods:** Fifty-three subjects occupationally exposed to powdered phytase from *Aspergillus niger* were studied. Exposure data and symptoms were registered by the company physician.

**Results:** Thirty-eight subjects complained of work-related respiratory symptoms and 14 of them showed phytase-specific IgE antibodies; only one asymptomatic subject revealed such antibodies. IgE antibodies were significantly more frequently found in the high-exposure group (technical centre) when compared with the low-exposure group (laboratories, experimental animal husbandry). Phytase-specific IgG antibodies were present in 19 symptomatic (50%) and five (33%) asymptomatic subjects.

**Conclusions:** Our results demonstrate that powdered fungal phytase is a highly sensitizing substance whose inhalation exposure should be avoided. Hypersensitivity symptoms could be prevented by means of extensive hygienic measures and ongoing medical surveillance.

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Phytase is an enzyme (phosphatase) that increases the bioavailability of phosphorous of plant origin in animal feeds. It catalyzes the hydrolysis of phytate (myoinositol - 1, 2, 3, 4, 5, 6-hexakisphosphate) to lower order phosphate esters and to inorganic phosphate. Phytate present in cereals and soy as a phosphate storage mode can only be partially utilized by pigs and poultry. Therefore fungal phytase is added to feed for pigs and poultry (monogastric animals). This supplementation of animal feed results in a phosphate release rendering a reduction of inorganic phosphate admixture possible and an increased bioavailability of chelated minerals such as magnesium or zinc (mineral-phytate chelates are insoluble at physiological pH) as well as of amino acids and proteins (1). In this way, the faecal phosphorous excretion and phosphate load of soils are reduced. Recently Doekes et al. (2) reported on sensitization to phytase in several workers of a factory producing animal feed additives. We noticed allergic symptoms such as dermatitis, conjunctivitis, rhinitis and asthma in some employees from a technical centre where phytase from *Aspergillus niger* was confectioned (3). Therefore we started a cross-sectional study whose aim was to assess the pathophysiological background of these diseases suspected of having an allergic mechanism.

## Material and methods

### Study group

We examined 53 employees occupationally exposed to phytase (14 women, 39 men, average age  $38 \pm 10$  years). Using a questionnaire, we registered and evaluated exposure data and symptoms (4) induced by phytase. The subjects were exposed to phytase dust during large-scale confection of phytase in powdered form in a technical centre, while analysing this enzyme in two laboratories or working in an experimental animal husbandry. All subjects underwent a personal interview by the company physician and were divided into a group with high exposure (subjects from the technical centre) and a group with low exposure (the others). We performed serological investigations of the phytase-specific IgE and IgG by means of EAST or ELISA, respectively. Immunoblots of the enzyme with pooled sera of five sensitized as well as five non-sensitized employees were also carried out.

### Material

*Aspergillus niger* phytase (myoinositol-hexakisphosphate 3-phosphohydrolase B; EC 3.1.3.8; 467 amino acids, molecular weight 51 028 Da; (BASF, Ludwigshafen, Germany) in powder form was used for each investigation. It contained 0.325 mg protein/mg substance (Bradfort).

### Phytase deglycosylation

The chemical deglycosylation of *Aspergillus niger* phytase was carried out under a controlled gas atmosphere with trifluoromethane

sulfonic acid in anisol, shaking in ice-cold diethyl ether and in an ice-cold mixture of pyridine and water (1 : 1). The subsequent dialysis was performed with an ammonium hydrogen carbonate solvent of 0.01%.

EAST (Enzyme-Allergo-Sorbent-Test)

Cyanogen bromide-activated discs were coated with the allergen diluted in sodium hydrocarbonate (1 mg/ml). The allergen discs were incubated with patients' sera for 3 h at room temperature followed by incubation with monoclonal antihuman-IgE labelled with alkaline phosphatase (Allergopharma, Reinbek, Germany) overnight at room temperature. For evaluation, a standard curve was used. IgE values of > 0.35 kU/l were classified as positive (RAST class 1 : 0.35–0.7 kU/l, RAST class 2 : 0.7–3.5 kU/l, RAST class 3 : 3.5–17.5 kU/l).

1-D gel electrophoresis (SDS-PAGE) and immunoblotting

SDS-PAGE was performed with 10% NuPAGE gel with 2-(N-morpholino) ethanesulfonic acid (MES) running buffer at 200 V for 42 min.

Blotting was performed on a polyvinylidene difluoride (PVDF) membrane (pore width 0.45 µm), 60 mA, 90 min. Pool sera each consisted of five IgE-positive and five IgE-negative sera of phytase-exposed employees. Dilution was 1 : 10. The antihuman IgE antibody was labelled with alkaline phosphatase. The development occurred with 5-bromo-4-chloro-3-indolyphosphate disodium salt and p-nitrotetrazolium chloride.

IgG-ELISA

Microtitre wells were coated with phytase using 2 µg protein per well. Dilution of patients' sera was 1 : 1000. In addition to subjects, we also tested five non-exposed people (negative controls). The detection was performed with antihuman IgG antibody labelled with alkaline phosphatase (A-3150; Sigma, Taufkirchen, Germany).

Results

Thirty-eight out of the 53 examined subjects complained of workplace-related dyspnoea (n = 12), rhinitis

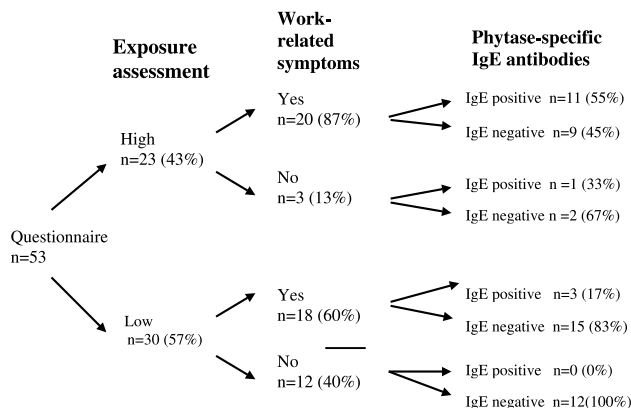


Figure 1. Study group, symptoms and results of phytase-specific IgE measurements.

(n = 35), conjunctival (n = 16) and/or cutaneous (n = 3) hypersensitivity reactions. In the highly exposed group, respiratory (87%) and conjunctival symptoms (43%) were more frequently recorded than in the group with low exposure (P = 0.031) (Fig. 1).

Fourteen out of 38 symptomatic subjects showed phytase-specific IgE antibodies. Such antibodies were only found once among the 15 subjects without complaints (P = 0.028). The frequency of IgE-positive subjects was 52% in the high-exposure group and 10% in the group with low exposure (12/23 vs. 3/30; P = 0.001). However, there was no significant correlation between the levels of IgE antibodies and the exposure degree of IgE antibody carriers (Fig. 2).

Nineteen out of 38 (50%) symptomatic subjects and five out of the 15 (33%) asymptomatic subjects showed phytase-specific IgG antibodies (Fig. 3). IgG antibody levels were not correlated with the degree of exposure nor was there a correlation between phytase-specific IgG and IgE antibodies (r = 0.011).

IgE-positive patients showed in immunoblots mainly IgE binding to a c. 50 kDa protein of deglycosylated phytase, which obviously represents *Aspergillus niger* phytase (see panel 0 in Fig. 4).

Minor IgE binding to proteins of c. 40 and 30 kDa was also seen.

When the before-mentioned test results were obtained extensive hygienic measures involving local exhaust systems, wearing of all protective clothing and masks with P 2 filters by all employees was implemented. After 2–8 weeks none of the workers reported workplace-related hypersensitivity symptoms when undergoing a second personal interview by the company physician.

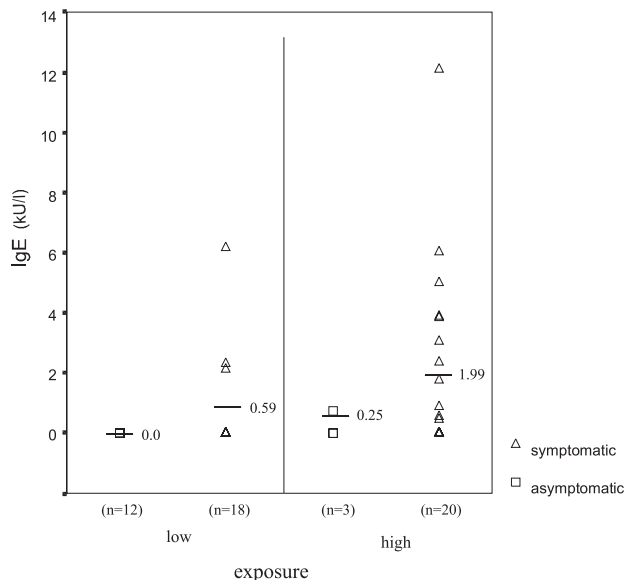


Figure 2. Phytase-specific IgE antibodies of symptomatic and asymptomatic workers with different exposure degrees.

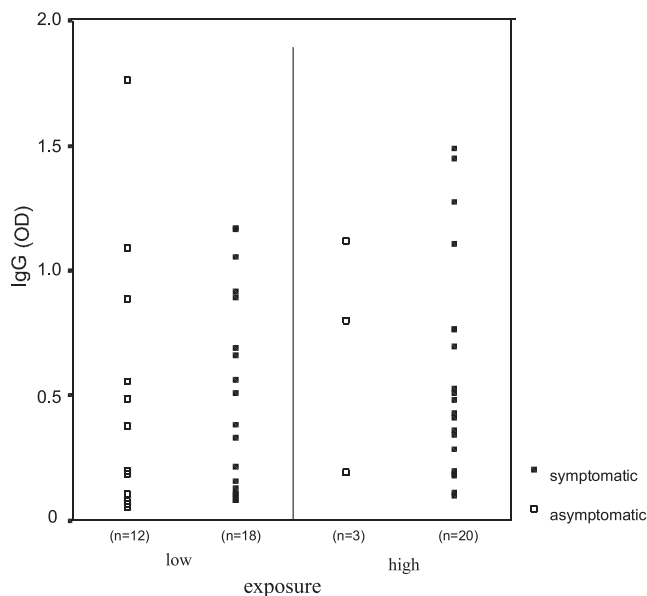


Figure 3. Phytase-specific IgG antibodies of symptomatic and asymptomatic workers with different exposure degrees.

## Discussion

From our results with 53 individuals handling powdered phytase in a technical centre, in laboratories or an experimental animal husbandry, 72% developed symptoms and 28% exhibited IgE-mediated sensitization. In contrast to phytase-specific IgE antibodies, corresponding IgG antibodies did not clearly distinguish between symptomatic and asymptomatic nor between low and highly exposed individuals. From these results we assume this enzyme to be a high risk factor for occupational asthma and rhinitis in the agricultural industry, where its use has increased in recent years. Corresponding data on this industry's population are not available yet.

Recently, O'Connor et al. (5) reported on an employee of an animal feed production plant who was sensitized to phytase and  $\beta$ -glucanase. Both powdered enzymes

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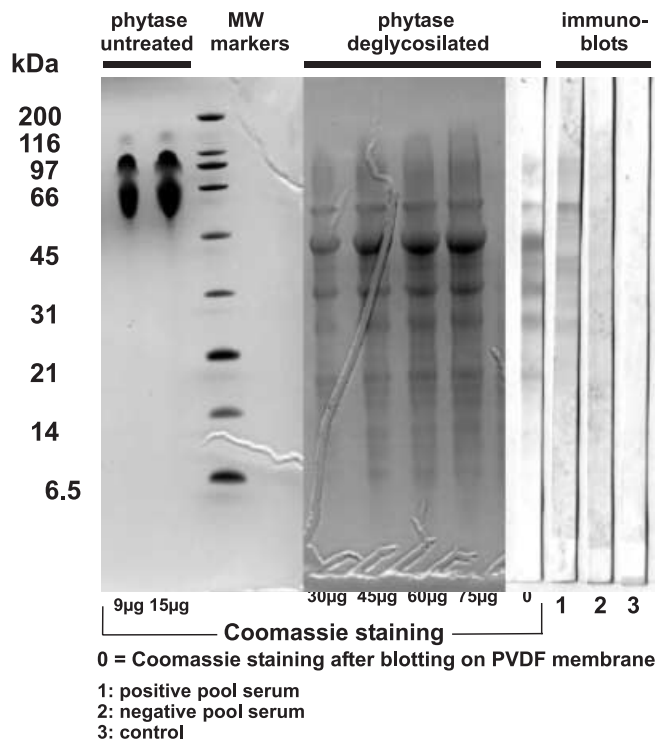


Figure 4. SDS-PAGE and immunoblotting of *Aspergillus niger* phytase. For details see text.

(sources were not given) produced immediate asthmatic reactions in inhalation challenge tests. Twenty-two other workers of the same plant had no symptoms.

We conclude that airborne phytase has a highly sensitizing potential. Direct contact with the enzyme in powder form should be prevented by corresponding protective measures, e.g., by the development of liquid or granulated products not producing dust. It could be demonstrated that local exhaust systems and wearing all protective clothing and masks are effective in secondary prevention.