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INCREASE IN TUMOUR NECROSIS FACTOR-α AND A CHANGE IN THE LACTATE DEHYDROGENASE ISOENZYME PATTERN IN PLASMA OF WORKERS EXPOSED TO AFLATOXIN-CONTAMINATED FEEDS

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Six types of animal-feed ingredients and swine mixed feeds from factories in northern Thailand were sampled for analysis of mycotoxins. Mycotoxins found in foodstuffs included aflatoxins, fumonisins, ochratoxins, T-2 toxin, vomitoxin and zearalenone. Samples of airborne dust generated while handling animal feed were collected and analysed to assess exposure of workers to aflatoxins. The average aflatoxin level in the control air samples was 0.99 ng/m^3. Higher levels of aflatoxins were found in the air samples taken by samplers attached to five workers adding hydrated sodium calcium aluminosilicate to animal feed (group 1; 1.55 ng/m^3) and five workers adding glucomannan to animal feed (group 2; 6.25 ng/m^3). The exposed workers showed a change in lactate dehydrogenase isoenzyme activity and tumor necrosis factor- levels in plasma. These changes may be associated with inhalation of mycotoxins and other contaminants in foodstuff. Occupational exposure to mycotoxins and mycotoxin adsorbents needs further evaluation in order to set up a proper system for long term surveillance of exposed population.

Key words:
air sampling, animal feed, chemosorbent, ELISA, mycotoxins, occupational exposure

Mycotoxins are natural products of fungi that may be present in food. Several mycotoxins have been associated with human and animal diseases (1). Diseases caused by mycotoxins are called mycotoxicoses, and are specific to the mold species and the toxin present.

Many mycotoxins with different chemical structures and biologic activities have been identified (2). The Food and Agriculture Organization estimates that 25% of the world's food crops are affected by mycotoxins. Aflatoxins have received greater attention than any other mycotoxin because of their potent carcinogenic effect demonstrated in susceptible laboratory animals and because of their acute toxic effect in humans (3, 4). Aflatoxin is significantly more common in warm and humid climates (5), although it may occur in temperate and cooler climatic areas. In tropical countries such as Thailand, warm and humid environment and inadequate storage of food may lead to extensive toxigenic mold growth and aflatoxin production (6).

Humans and animals can be exposed to aflatoxin in many ways (7). The main route of exposure is consumption of aflatoxin-contaminated food or feed (2), but another increasingly recognised route is the inhalation of fungal spores (8) or of grain dust, as evidenced by significant amounts of aflatoxins found in respirable particles of the latter (9, 10). Several studies of animal exposure (such as mice, guinea pigs, hamsters, or rats) to aflatoxins or aflatoxin B1 (AFB1) in aerosol showed extensive pulmonary pathology (11). Genotoxic effects (12), and increased lymphatic leukemia (13) or suppression of alveolar macrophage phagocytosis (14) were also observed.

Because aflatoxin contamination of food cannot be avoided, numerous detoxification strategies have been proposed to alleviate its impact. A new approach to the detoxification of aflatoxins involves addition of chemisorbents such as phyllosilicate clay, bentonite, and zeolite to the animal diet. Hydrated sodium calcium aluminosilicate (HSCAS) markedly reduced the toxicity of aflatoxin (15-18), but has little effect on ochratoxin A (19), cyclopiazonic acid (20), and T-2 toxin (21). Hydrated sodium calcium aluminosilicate can alleviate some of the reproductive effects of zearalenone which are not related to estrogenic action (22). It has the ability to tightly bind and immobilise aflatoxins in the gastrointestinal tract of animals, substantially reducing their bioavailability (17).

Most information about mycotoxin toxicity comes from studies of experimental animals, which may not reflect the true mycotoxin exposure of humans and non-experimental animals. This study investigated the actual human exposure to aflatoxins in contaminated animal feed collected from feed factories in northern Thailand. We used passive dosimetry to measure occupational mycotoxin exposure of workers mixing feeds. We further investigated two animal feed chemosorbents common in Thailand, that is, HSCAS and glucomannan, to see how effective they are in reducing mycotoxin airborne dust concentrations. The toxic effect of aflatoxins on exposed workers was measured through plasma tumor necrosis factor-alpha (TNF-α) and the activities of plasma lactate dehydrogenase (LDH) isoenzymes and the results were compared with control.

SUBJECTS AND METHODS

The study included two exposed groups of subjects (N=10) who mixed feed in one animal feed factory in northern Thailand. Group 1 consisted of five subjects mixing swine feed

with HSCAS, and Group 2 of five subjects finishing swine feed by adding glucomannan. The control group consisted of four employees who did not mix feed. All subjects gave their informed consent prior to inclusion in the study. Before the investigation started, we completed a questionnaire with basic personal and job-related data (Table 1).

Table 1 *Characteristics of 14 workers*

	Exposed workers		**Control (N=4)**
	Group 1 (N=5)	**Group 2 (N=5)**	
Average age in years (range)	22 (18-27)	32 (30-35)	36 (25-52)
Sex (male/female)	5/0	5/0	2/2
Months of employment (mean)	1	11	>12
Non-smokers (N)	1	0	3
Smokers (N)	4	5	1

The feed sampling involved six types of animal feed ingredients and swine mixed feeds from factories in northern Thailand. Enzyme-Linked-Immuno-Sorbent Assay (ELISA) (Thai-Neo Biotech Co., Ltd.,Thailand) was used to detect mycotoxins in samples.

Passive dosimetry was used to measure occupational exposure by inhalation of workers mixing mycotoxin-contaminated feeds. The exposed and control workers wore personal samplers (Universal PCXR samplers; model 224-44XR) with the flow rate of 2,000 ml/min for 2-8 hours. The recording was taken once. The ELISA kit (Thai-Neo Biotech Co., Ltd.) was then used to determine the content of aflatoxins in sampled dust.

Venous blood was collected in clean tubes containing heparin and centrifuged at 1000 x g for 10 minutes. Plasma samples were then separated and stored at -20 °C until analysis.

Plasma tumor necrosis factor alpha was quantified by ELISA using a commercial kit (Diaclone Co., Ltd.) according to the manufacturer's instructions.

Lactate dehydrogenase (LDH) isoenzymes were determined using the method of Helena laboratory (23). Plasma samples were applied on the cellulose acetate plate as a carrier used for electrophoretic separation. Tris-barbital buffer solution and LDH isoenzyme reagent were used for staining. The activity of each isoenzyme determined by scanning densitometer.

The data are presented as mean values±S.E. The significance of difference was tested by the Student's *t*-test or by one-way analysis of variance. Differences were considered significant where $P<0.05$.

RESULTS

The content of mycotoxins in animal feed ingredient and mixed feed for swine

Six common mycotoxins, that is, aflatoxins, fumonisins, ochratoxins, T-2 toxin, vomitoxin, and zearalenone were detected in animal feeds in different amounts (Table 2). Rice bran was highly contaminated with 43.9 ng/kg of total aflatoxins, which is substantially over

the allowed US FDA limit for animal feed. A high level of zearalenone was found in cassava mill (768.2 ng/kg). Fumonisins, ochratoxins, T-2 toxin and vomitoxin concentrations kept within US FDA limits in all samples.

Table 2 *Mycotoxin content in animal feeds and feed ingredients*

	Aflatoxins (ppb)	Fumonisins (ppm)	Ochratoxins (ppb)	T-2 Toxin (ppb)	Vomitoxin (ppm)	Zearalenone (ppb)
Feed ingredients						
Soybean mill	9.2	0.0	12.1	46.8	0.6	119
Extruded soybean	5.4	0.0	13.0	< LOD	0.0	0.0
Cassava mill	0.0	0.3	< LOD	0.0	0.3	768
Fish mill	< LOD	< LOD	< LOD	44.6	0.0	0.0
Broken rice	< LOD	0.3	16.6	0.0	< LOD	0.0
Rice bran	43.9	0.0	< LOD	0.0	0.5	76.6
Swine mixed feed						
Factory I	8.6	0.0	23.0	0.0	< LOD	109
Factory II	10.6	0.0	18.1	0.0	0.6	200
Factory III	3.1	< LOD	9.4	< LOD	0.9	387

< LOD Below the limit of detection

Occupational exposure to contaminated feed dust

Aflatoxin was found in all three groups of subjects. However, samples collected from the control group contained lower levels of aflatoxin (0.99±0.74 ng/m^3) than samples collected from the two exposed groups (group 1: 1.55±1.22 ng/m^3 and group 2: 6.25±2.48 ng/m^3).

Plasma biomarkers

A difference in LDH activities (Table 3) in plasma was observed between the exposed workers and reference values/controls. The heart isoenzyme activity LDH1 decreased, whereas the spleen and lung isoenzymes LDH3 and LDH4, significantly increased in both exposed groups ($P<0.05$). There were no significant differences in LDH2 and LDH5 activities.

Table 3 *Activities of lactate dehydrogenase (LDH) isoenzymes in three groups of subjects*

Group	LDH1	LDH2	LDH3	LDH4	LDH5
Helena Laboratories reference range of normal value	24.6-29.80	31.8-35.80	20.0-24.80	6.8-10.20	6.5-9.70
Control (N=4)	31.0±2.04	33.3± 0.85	20.8± 1.44	6.0± 0.82	8.8±0.95
Exposed workers group 1 (feed plus HSCAS)	15.8±1.32*	30.0±0.55	29.4±2.20*	14.0±1.14*	10.8±1.62
Exposed workers group 2 (feed plus glucomannan)	20.2±1.11*	33.6±1.75	29.8±2.01*	9.4±1.44*	7.2±1.50

Values are expressed as mean S.E.M.

* Significant difference from control groups by one-way ANOVA, followed by the Duncan's multiple range test at $P<0.05$

Higher plasma TNF-α levels were also found in both exposed groups (group 1, 86.6±29.7 pg/ml and group 2, 107.0±21.5 pg/ml), whereas none were detected in controls.

DISCUSSION

The results of this study revealed a certain level of mycotoxin contamination of animal feeds produced in northern Thailand. The mycotoxin levels varied between aflatoxins, fumonisins, ochratoxins, T-2 toxin, vomitoxin, and zearalenone. Some samples showed mycotoxin levels exceeding the US FDA recommendations.

Occupational exposure of workers to aflatoxins was established by sampling airborne dust generated in the mixing process of animal feed production using individual monitoring samplers which showed positive reaction of antibodies to aflatoxins. These findings indicate that dust generated in the process of mixing contaminated feed bears potential inhalation hazard. Appropriate measures should be taken to reduce occupational exposure to contaminated materials.

It is common that animal feeds are added various adsorbents which help to reduce mycotoxin exposure of the livestock. Our study suggests that HSCAS is more effective than glucomannan in reducing aflatoxin levels in feed and presumably their effect on humans.

Hydrated sodium calcium aluminosilicate and other phyllosilicate clay show no toxicity through the oral route of exposure (16). *Abdel-Wahhab and co-workers* (15) showed that aluminosilicate and bentonite added to diet (0.05%) and fed to pregnant rats throughout pregnancy had no maternal or developmental toxicity. However, the toxic effects of these compounds when inhaled have not yet been evaluated.

An elevation in extracellular LDH activity in body fluids such as serum indicates cell damage and lysis (24). Total LDH activity is non-specific, whereas isoenzyme patterns can indicate the injured organ or tissue (25-27). Changes in LDH isoenzyme activities in serum have been previously observed in dogs after pulmonary embolism (increase in LDH3) (27), in mice after immunologically-induced lung injury (increase in LDH2 and LDH3) (28), and chemically-induced airway injury (increases in LDH2-4) (29).

Our results show a significant change in LDH isoenzyme profiles in workers exposed to aflatoxin in feed dust, even though glucomannan and HSCAS were added to it. A significant increase was observed in the activities of LDH3 and LDH4, which are major isoenzymes found in the lung and the spleen. The significant increase in plasma TNF-α levels correlated with changes in plasma LDH profiles. These results suggest that the damage of the lung and spleen cells may be associated with occupational exposure to aflatoxin through inhalation of contaminated dust.

TNF-α is a bioactive mediator produced by keratinocytes, dermal dendritic cells, macrophages and lymphocytes that can mediate a variety of biologic effects. Cellular effects of TNF-α include physiologic, cytotoxic, and inflammatory processes (31, 32). In homeostasis, TNF-α influences mitogenesis, differentiation, and immunoregulation while causing apoptotic cell death in some neoplastic cell lines or tumour growth in other cells.

Cytotoxicity by TNF-α occurs independently of *de novo* transcription and translation and involves mitochondrial production of oxygen radicals. This requires ceramide, a sphingolipid generated in cells following stimulation with TNF-α, which generates H_2O_2 from the mitochondrial electron transport chain. Recently, the expression of LDH-A mRNA in porcine cultured sertoli cell induced by TNF-α has been demonstrated (33).

Chronic inflammatory lung diseases, such as idiopathic pulmonary fibrosis, chronic bronchitis, cystic fibrosis, and some forms of asthma, are associated with elevated TNF-α responses and neutrophil accumulation in the lung. Increased levels of TNF-α have also been found in bronchoalveolar lavage fluid following inhalation of environmental agents associated with pulmonary inflammation or fibrosis (silica, asbestos) (34, 35).

Plasma TNF-α and LDH-profile changes in our study may be the result of chronic inhalation of low-dose aflatoxin in feed dust and chemosorbent particles added in the feed. However, the effect of other mycotoxins, though present in traces, could not be excluded. Further considerations of health hazards should focus on the most appropriate marker(s) for monitoring occupational/respiratory exposure to mycotoxins of farmers, mixing workers, millers, and truckers. Moreover, the use of chemosorbents to protect animals from adverse effects of mycotoxins should be more cautious until proven harmless when inhaled.

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Sažetak

POVIŠENJE KONCENTRACIJE TUMORSKOGA NEKROTIZIRAJUĆEG FAKTORA-α I PROMJENA AKTIVNOSTI ENZIMA LAKTAT DEHIDROGENAZE U RADNIKA EKSPONIRANIH PRAŠINI STOČNE HRANE ONEČIŠĆENE MIKOTOKSINIMA

U ovome radu autori su analizirali mikotoksine u uzorcima 6 vrsta mješavina hrane za svinje iz Sjeverne provincije u Tajlandu, a sa svrhom procjene ekspozicije radnika prašini kontaminiranoj mikotoksinima. Pratili su i neke biološke učinke u 10 eksponiranih radnika i u 4 neeksponirana, koji su služili za usporedbu. U uzorcima hrane za svinje utvrdili su prisutnost aflatoksina, fumonizina, okratoksina, T-2-toksina, vomitoksina i zeralenona. Uzorci udisanog zraka skupljani su individualnim sisaljkama za uzorkovanje, i to tijekom 8-satnog rada i uz protok od 2.000 ml/min. Aflatoksine u skupljenim uzorcima zraka analizirali su s pomoću ELISA kita. Prva skupina od 5 radnika bila je eksponirana prašini iz mješavine stočne hrane kojoj je prethodno dodan adsorbens aluminijev silikat. Uzorak je sadržavao 1,55 ng aflatoksina u m^3 usisanog zraka. Drugih 5 radnika koji su radili sa stočnom hranom kojoj je prethodno primiješan adsorbens glukomanan bila je eksponirana prašini što je sadržavala 6,25 ng aflatoksina u m^3 udisanog zraka. U uzorcima udisanog zraka neeksponiranih osoba bilo je 0,99 ng aflatoksina u m^3. Aktivnost izoenzima LDH u plazmi određivana je elektroforezom. U eksponiranih radnika aktivnost LDH1 bila je značajno ($P<0,05$) niža, a LDH3 i LDH4 značajno ($P<0,05$) viša negoli u neeksponiranih radnika. Koncentracija tumorskoga nekrotizirajućeg faktora-α određivana je s pomoću komercijalnog ELISA kita i iznosila je 29,7 pk/ml plazme u radnika prve skupine i 107 pk/ml plazme u radnika druge skupine. U neeksponiranih radnika ovaj se faktor nije mogao detektirati. Dobiveni rezultati upućuju na vjerojatnost da bi udisanje prašine kontaminirane aflatoksinom, a možda i drugim mikotoksinima, moglo dovesti do oštećenja ciljnih organa, vjerojatno pluća i slezene.

Ključne riječi:
ELISA, kemijski adsorbensi, profesionalna izloženost, stočna hrana, uzorkovanje zraka

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