Effects of Dietary Supplementation of Fermented Chitin-chitosan (FERMKIT) on Toxicity of Mycotoxin in Ducks

J. M. Khajarern*, S. Khajarern, T. H. Moon[1] and J. H. Lee[1]
Department of Animal Science, Khon Kaen University, Khon Kaen 40002, Thailand

ABSTRACT : Two experiments were conducted to evaluate the efficacy of dietary FERMKIT, a commercial toxin binder consisting of probiotic-fermented natural product containing chitin, chitosan and chitosan oligosaccharides (FERMKITO®, EASY-BIO SYSTEM, Inc., Korea), in binding aflatoxin (AF) and zearalenone (ZEN) and ameliorating their mycotoxicity in meat type ducks. FERMKIT was supplemented to AF contaminated diets (at 120 ppb) at either 0.3 or 0.6% in experiment 1 and to ZEN contaminated diets (at 150 ppb) at 0.6% in experiment 2. In experiment 1 body weight gains were reduced by 37% and mortality was increased by 18% in ducks fed diet contaminated with AF at 120 ppb compared to ducks fed control diet (<10 ppb AF) for the 4-wk experimental period. However, dietary FERMKIT supplementation effectively alleviated overall toxicity induced by AF. The significant treatment-related changes in feather growth, web-toe hemorrhage, leg deformity, liver paleness, organ weights, hematological values and serum biochemical values, as compared to the control, were observed. The FERMKIT supplementation significantly diminished the adverse effects of AF and restored all the parameters measured back (<0.05) toward the control values. These findings indicated that FERMKIT, when added at the levels of 0.3 or 0.6% in the 120 ppb AF diets, could modulate the toxicity of AF with percentage sorption capacity of 52.70% at the level 0.3% and 79.85% at the level 0.6% of the diets (experiment 1). In experiment 2, FERMKIT, when added at 0.6% to the 150 ppb ZEN diets for the 4-wk experimental period, diminished the toxicity as shown by body weight gain, weights of testicles, oviducts, Bursa of Fabricius and cloaca eversion score as compared with the controls (<10 ppb ZEN) and 150 ppb ZEN diet with no added FERMKIT. The findings indicated that FERMKIT could be protective against the effects of ZEN in young growing ducks with percentage sorption capacity of 67.11% as evaluated from toxicity index parameter measured when added at 0.6% of the diets containing 150 ppb ZEN. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 5 : 706-713)***

Key Words : Duck, Mycotoxicity, Aflatoxin, Zearalenone, Fermented Chitin-chitosan

INTRODUCTION

Mycotoxins

Mycotoxins are found in most agricultural products. Peanuts (groundnut) and corn are frequently associated with mycotoxin contamination, especially aflatoxin, which can occur either pre or post harvest.

Aflatoxins (AF) are produced by *Aspergillus flavus* and *Aspergillus parasiticus* and are potent hepatotoxin possessing carcinogenic activity. A positive association between aflatoxin ingestion and liver cancer in man has been found in many population studies in tropical regions of Africa, India, South East Asia and the Philippines (WHO, 1989). There are a number of comprehensive reports on aflatoxicosis and biological of aflatoxin (Baird, 1978; Wogan and Busby, 1980; Applebaum and Marth, 1982; Bryden, 1982). The symptoms of aflatoxicosis in poultry ranged from none to acute or chronic toxicity and varied with the species of bird, the amount of toxin consumed and the length of time over which it was ingested. The duckling is very sensitive to AF. The clinical signs are not very definite but include loss of appetite, retard growth, reduced feed efficiency, haemorrhage in the webs of toes, ataxia, malabsorption syndrome, steatorrhea or failure to utilize lipids, impaired protein utilization, appearance of nervous symptoms, high and rapid mortality in young birds and lie on their side with opisthotonus shortly before death (Khajarern et al., 1990). Hematologically, aflatoxicosis caused anemia as noted by significant decreases of hemoglobin, hematocrit, erythrocyte count and mean corpuscular volume. Absorption and retention of macrominerals is also reduced (Mohiuddin et al., 1986). Immune system is generally suppressed by causing atrophy of Bursa of Fabricius, thymus and spleen and abnormally increase of circulating lymphocyte (Richard et al., 1978).

Zearalenone (ZEN) or F-2 toxin occurs as natural mycotoxin contaminant in corn, wheat, barley, oats and sorghum (Mirocha et al., 1971, 1977, 1982; Krogh, 1976). ZEN is produced by strains of *Fusaria (F. avenaceum, F. equiseti, F. gravinearum, F. culmorum, F. lateritium).* A period of low temperature and alternating moderate to low temperature variations is required for significant toxin formation. The average contamination rates within different countries ranged from 1 ppb to 10.1 ppm. ZEN is an estrogenic mycotoxin (beta-resorcylic acid lactone) which generally causes vulvovaginitis, prolapse of vagina (accumulation of interstitial edema causes thickness of vulva, vagina, cervix and myometrium), grossly enlarged uterine horns, mammary glands and frequently nipples, swollen and reddened vulva in young animal. Swine are the

* Reprint request to: J. M. Khajarern. Tel: +66-43-241733, Fax: +66-43-244419, E-mail: jowaman@kku.ac.th
[1] Easy-Bio system, Inc., Korea.
Received September 2, 2002; Accepted January 8, 2003

most commonly affected animals. Cattle, poultry and laboratory rodents are also affected, but to a lesser degree. ZEN has been associated with feminization in young male swine, including testicular atrophy, swollen prepuce and mammary gland enlargement. Decreased libido may be a variation sequela, but mature boars apparently have enough testicular reserve to avoid decreased spermatogenesis. Abortion and mummified piglets and spread leg syndrome to a ZEN toxicosis have also been reported (Bacon and Marks, 1976; Kurtz and Mirocha, 1978; Allen et al., 1981a, 1981b; CAST, 1989; Danicke, 2001). In poultry, toxicosis causes vent enlargement, cloacal eversion, mucous secretion in feces, cystic changes in the oviduct and enlargement of the Bursa of Fabricius (Wyllie and Morehouse, 1977; Chi et al., 1980a and b; Dailey et al., 1980; Allen et al., 1981a and b; Miercha et al., 1982; Maryamma et al., CAST, 1989, 1992; Wood, 1992).

Detoxification of Mycotoxins

Numerous strategies for the detoxification of mycotoxin containing food and feed have been reported (Palmgren and Hayes, 1987; Ramos, 1994; Ramos et al., 1996; Danicke, 2001). There are considerable informations, both theoretical and practical, on the detoxification of aflatoxins; however, information on detoxification of other mycotoxins is limited (CAST, 1989). Prevention and control of mold growth and detoxification can be achieved by treating feeds or ingredients physically, chemically, biologically and naturally by products from plants or herbs (Suttajit, 1989). Sequestrant or chemisorbents (inorganic sorbent materials: zeolite, bentonite, clay, hydrated sodium calcium aluminosilicates, activated charcoal) have been shown to alter the effects of aflatoxin in chickens, (Phillips et al., 1987, 1988, 1990, 1994; Harvey et al., 1989, 1993; Kubena et al., 1990a, b, 1991, 1993; Davidson, 1987; Ellis et al., 1990, 1991; Chung et al., 1990; Huff et al., 1992). However, the optimum inclusion rates of these chemisorbents are generally high, for instance 5-10% for activated charcoal, and consequently, other essential nutrients, vitamins, antibiotics or growth promoters are also adsorbed causing even more negative impact on animal performance. Among all aluminosilicates tested with regard to mycotoxin absorption, a hydrated sodium calcium aluminosilicate (HSCAS) from natural zeolite has been most extensively studied because of its promising aflatoxin-binding capacity (Teng, 1974, Ramos et al., 1996). Due to the promising results obtained with aflatoxin, the addition of HSCAS was assayed with variety of mycotoxins, including ZEN (Bursian et al., 1992) and ochratoxin A (Huff et al., 1992) but its efficacy against ZEN and ochratoxin A was limited (Ramos et al., 1996).

For other absorbent compounds, Cuero et al. (1988) used chitosan or derivative of chitin isolated from crustacean shell as a control agent of toxicogenic fungal growth and aflatoxin production. Khajarern et al. (2001) demonstrated, by using growth rate, feed efficiency, liver weight and liver paleness score as tested criteria, that chitin-chitosan powder (60 mesh with 75.6% degree of deacetylation), when added at 0.05 and 0.10% of the diets for 0-3 week-old meat-type ducks (Cherry Valley) with 120 ppb total aflatoxin concentration as compared with HSCAS (Novasil^{TM}, Engelhard Corp., Cleveland, OH), was able to absorb AF upto 19% for the 0.05% and 33% for the 0.10% inclusion levels.

The objective of the present study was to evaluate the efficacy of FERMKIT, a probiotic-fermented natural product of chitin, chitosan and chitosan oligosaccharide (FERMKITO$^{®}$, EASY-BIO SYSTEM, Inc., Korea), for protection against the toxicity of AF and ZEN in meat-type duck's diets.

MATERIALS AND METHODS

Two experiments were conducted at Khon Kaen University's Poultry Research Farm. Experiment 1 was to evaluate the effects of dietary FERMKIT for its ability to bind and ameliorate aflatoxicosis in meat-type ducks (Cherry Valley) and Experiment 2 for ZEN. Just prior to the beginning of the experiments, samples of corn, broken rice, rice bran, soybean meal and fish meal were analyzed for total AF content by method of Dicken et al. (1980) and ZEN by AOAC (1995) method using high pressure liquid chromatography. Aflatoxin used was produced through the fermentation of corn by *Aspergillus parasiticus* as was previously described by Shotwell et al. (1966) and further modified by West et al. (1973). For the ZEN trial (Experiment 2) soybean meal with the Fusarium toxins (590 ppb ZEN) contamination ("Fusarium-year 2001 in Thailand") was incorporated into the diets by replacing good quality source of soybean meal in the control diet with an appropriate amount of the contaminated soybean meal to provide the given ZEN concentration in the final test diet.

Experiment 1

One-day-old meat-type ducks (Cherry Valley) were randomly distributed into each of six dietary treatments. Those are: <10 (control), 30, 60 and 120 ppb AF or 120 ppb AF diets supplemented with FERMKIT at 0.3 or 0.6%. Each treatment contained 2 replications (pen) of 25 birds each. All birds were housed in concrete-floored pens under continuous lighting throughout the 4-week duration of the experiment and were given feed and water *ad libitum*. The composition of the experimental rations are given in Table 1. The birds were clinically observed at least twice daily and mortality was recorded. Group weight by pen were recorded on a weekly basis and feed consumption was recorded

Table 1. Composition of the basal diets (0-4 wk)

Ingredients, %	Experiment 1				Experiment 2	
	Control	AF, ppb			Control	ZEN, ppb
	<10	30	60	120	<10	150
Broken rice	10.00	10.00	10.00	10.00	10.00	10.00
Corn, grade # 1	38.50	29.50	20.50	2.50	38.50	38.50
Corn with AF 300 ppb	-	9.00	18.00	36.00	-	-
Rice bran	6.80	6.80	6.80	6.80	6.80	6.80
Soybean meal (44% CP)	28.00	28.00	28.00	28.00	28.00	2.00
Soybean meal with ZEN 590 ppb	-	-	-	-	-	26.00
Fish meal (60% CP)	10.00	10.00	10.00	10.00	10.00	10.00
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50
Limestone	0.10	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.23	0.23	0.23	0.23	0.23	0.23
L-Lysine	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.18	0.18	0.18	0.18	0.18	0.18
Rice bran oil (crude)	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin-mineral premixes[1]	0.50	0.50	0.50	0.50	0.50	0.50
Calculated analysis						
Protein, %	23.00	23.00	23.00	23.00	23.00	23.00
ME, Kcal/kg	3,169	3,169	3,169	3,169	3,169	3,169
Chemical analysis						
Protein, %	22.84	22.80	22.78	22.88	22.81	22.89
Total AF, ppb	4.6	31.6	61.8	122.0	3.6	4.9
Total Zen, ppb	7.6	8.2	7.9	9.2	9.6	152.8

[1] Vitamin-mineral premixes provide per kg of diet: vitamin A 5,500 IU; vitamin D, 2,500 IU; vitamin E, 5 IU; vitamin K, 1.45 mg; thiamin, 20 mg; riboflavin, 7.0 mg; pyridoxine, 5.0 mg; vitamin B_{12}, 30 mcg; pantothenic acid, 12 mg; niacin, 50 mg; choline, 500 mg; folic acid, 1 mg; biotin, 15 mcg; Mn, 50 mg; Zn, 40 mg; Fe, 80 mg; Cu, 8 mg; Co, 1.5 mg; I, 0.35 mg; Se, 0.31 mg; ethoxyquin, 125 mg.

weekly. At the end of experiment, all birds that remained alive were examined on feathering condition, leg deformity and web-toe hemorrhage. Blood samples were collected from 20 birds (10 males and 10 females) from each treatment (using birds with body weight near the treatment mean body weight) for serum biochemical analyses and hematological determination. After bled, all birds form each treatment were killed by cervical dislocation and the liver, kidney, heart, spleen and pancrease were removed and weighed. Liver paleness and lipid content (AOAC, 1995) of liver were also determined.

Experiment 2

Three dietary treatments were used in Experiment 2: 1) control diet containing no added ZEN or FERMKIT; 2) diet contained 150 ppb ZEN no added FERMKIT; and 3) diet contained 150 ppb ZEN plus 0.60% FERMKIT. Each treatment also contained 2 replications (pen) of 25 birds each. All birds were also housed in concrete-floored pens under continuous lighting as in Experiment 1 and *ad libitum* access of water and feed. The composition of the rations fed are given in Table 1. Birds were also daily observed on mucous excretion, swelling of the vent, eversion of the cloaca, etc. Body weight gain and feed consumption were determined at weekly interval. At the end of 4 weeks experiment, all birds that remained alive were examined for eversion of the cloaca. The weights of oviducts or testicles and Bursa of Fabricius were determined on 10 females and 10 males form each dietary treatment. Necropsy was performed on all birds and they were examined for any abdominal clinical symptoms.

The data for all parameters were subjected to analysis of variance (SAS Institute Inc., 1989) and significant treatment differences were determined by Duncan's multiple range test (Duncan, 1955). The pooled standard errors which are calculated as the square root of the error mean square from the analyses of variance are reported. The statement of significance was based on the 0.05 level of probability.

RESULTS AND DISCUSSION

Experiment 1

Effect of dietary FERMKIT and AF on duck feed intake, mean body weight gain, feed efficiency (feed:gain), mortality and livability and economic loss index (ELI) which calculated from mean body weight gain and livability are given in Table 2. The AF adversely affected duck's feed intake, body weight gain, feed efficiency and mortality. Ingesting AF at the level of 120 ppb of diets caused significant ($p<0.05$) decreases in feed intake, mean body

Table 2. Effects of dietary FERMKIT and aflatoxin on duckling weight gain, feed efficiency, percentage of mortality and livability and economic loss index (ELI) (Experiment 1)

Dietary treatment		Feed intake, kg	Mean body weight gain, g	Feed conversion (g feed:g gain)	Mortality, %	Livability, %	Economic loss index*
Aflatoxin, ppb	FERMKIT %						
<10	-	1.77[a]	898[a]	1.97[b]	0.00	100.00[a]	898[a]
30	-	1.77[a]	877[a]	2.02[b]	2.00	98.00[a]	859[ab]
60	-	1.78[a]	831[a]	2.14[b]	4.00	96.00[a]	798[b]
120	-	1.58[b]	565[b]	2.79[a]	18.00	82.00[b]	464[c]
120	0.30	1.82[a]	896[a]	2.04[b]	2.00	98.00[a]	879[ab]
120	0.60	1.75[a]	890[a]	1.97[b]	0.00	100.00[a]	890[a]
SEM	-	0.06	40.21	0.07	-	2.06	31.79

[a-c] Means within a column with no common superscripts differ significantly ($p<0.05$)

* Economic loss index = mean body weight gain×livability×10^{-2}

weight gain, livability and poor in feed efficiency. Symptoms of anorexia, listlessness, dehydration, ataxia and lie on their side with opisthotonus shortly before death were observed with the 120 ppb AF. Similar toxicity signs have generally been observed and reported by several workers (Baird, 1978; Khajarern et al., 1990). Khajarern et al. (1990) noted that ducklings were very sensitive to aflatoxicosis and the toxicity signs including poor growth performance, high mortality, enlarged liver with high lipid content, poor feather growth or helicopter-wing disease and leg deformity began to show once duckling consumed diet contaminated with as low as 30 ppb of AF. Adding 0.3 and 0.6% FERMKIT to the diet containing 120 ppb AF diminished the adverse effects of AF on feed intake, mean body weight gain, feed efficiency, mortality and livability. The addition of FERMKIT caused a marked reduction of AF toxicity when compared with the control (<10 ppb) and the AF 120 ppb treatment without FERMKIT.

Impaired feather growth, web-toe hemorrhage, liver paleness and lipid content were significantly ($p<0.05$) increased in the ducks fed with graded levels of AF (Table 3). The addition of FERMKIT at the level of 0.6% to the 120 ppb AF diets resulted in total protection against aflatoxicosis as shown by significant ($p<0.05$) alleviation of the abnormal feather growth, leg deformity, web-toe hemorrhage, liver paleness and reduced liver lipid content to the normal level. Data in Table 4 demonstrated that aflatoxicosis caused significant hypertrophy ($p<0.05$) of liver, kidney, heart, spleen and pancrease. Added FERMKIT, at 0.6% of the diet, significantly diminished ($p<0.05$) all the adverse effects resulting in the reduced organ weight toxicity index (OWTI) to a normal level. These data agreed with the previous reports of Khajarern et al., (2001) who compared the AF absorbing ability of a HSCAS (0.5% of diet) with chitin-chitosan powder (60 mesh with 75.6% deacetylation) at 0.05 or 0.10% of duckling (0-3 weeks) diets contaminated with 120 ppb AF and revealed that chitin-chitosan could alleviate aflatoxicosis by improving body weight gain, feed conversion, survivability, weight and paleness score of liver to the degree comparable to those resulted by HSCAS. The AF absorbance index (feed conversion ratio×% survivability×liver weight in g) of chitin-chitosan at 0.05 and 0.10% inclusion were 138.61 and 80.87 as compared with 34.13 for the HSCAS and 26.79 for the control diet (<30 ppb AF). They indicated that chitin-chitosan was able to absorb AF upto 19% for the

Table 3. Effects of dietary FERMKIT and aflatoxin on duckling feather growth, web-toe hemorrhage, leg deformity, liver paleness and liver lipid (Experiment 1)

Dietary treatment		Feather growth score[1]	Leg deformity score[2]	Web-toe hemorrhage score[3]	Liver paleness score[4]	Liver lipid % dry wt.	Appearance chronic toxicity index*
Aflatoxin ppb	FERMKIT %						
<10	-	2.54[a]	0.32[d]	0.05[d]	1.07[d]	17.98[bc]	0.31[d]
30	-	2.16[b]	0.63[cd]	0.14[c]	1.32[cd]	18.48[bc]	2.07[cd]
60	-	1.78[cd]	1.63[b]	0.23[b]	1.94[b]	20.42[ab]	14.78[b]
120	-	1.55[d]	2.38[a]	0.92[a]	2.44[a]	22.77[a]	121.02[a]
120	0.30	1.96[bc]	0.88[c]	0.18[bc]	1.44[c]	17.39[c]	4.01[c]
120	0.60	2.20[b]	0.63[cd]	0.12[cd]	1.38[c]	17.15[c]	1.92[cd]
SEM	-	0.12	0.17	0.03	0.11	0.98	1.26

[a-d] Means within a column with no common superscripts differ significantly ($p<0.05$)

[1] Feather growth score: Range of 1 to 3, 1 = poor, 2 = moderate and 3 = good.

[2] Leg deformity score: Range of 0 to 4,0 = normal, 1 = one leg slightly deformed, 2 = both legs slightly deformed, 3 = one leg slightly deformed, the other severely deformed and 4=both legs severely deformed.

[3] Web-toe hemorrhage score: Range of 0 to 2, 0 = normal, 0.5 = slightly hemorrhage, 1 = moderately hemorrhage and 2 = severely hemorrhage

[4] Liver paleness score: Range of 1 to 3, 1 = normal, 2 = moderate and 3 = very pale.

* Appearance chronic toxicity index (ACTI) = Leg deformity score×web-toe hemorrhage×liver paleness×liver lipid.

Table 4. Effects of dietary FERMKIT and aflatoxin on duckling weights of liver, heart, kidney, spleen, and pancrease (Experiment 1)

Dietary treatment		Liver	Heart	Kidney g/100 g BW	Spleen	Pancrease	Organ weight toxicity index (OWTI)*
Aflatoxin ppb	FERMKIT %						
<10	-	3.20[c]	0.62[c]	0.74[c]	0.083[c]	0.302[b]	3.62[c]
30	-	3.74[c]	0.68[bc]	0.80[c]	0.094[b]	0.318[b]	6.04[c]
60	-	4.24[b]	0.74[ab]	0.93[b]	0.102[a]	0.338[b]	9.99[b]
120	-	5.57[a]	0.82[a]	1.14[a]	0.109[a]	0.432[a]	24.34[a]
120	0.30	3.54[cd]	0.70[bc]	0.82[c]	0.090[bc]	0.309[b]	5.64[c]
120	0.60	3.35[de]	0.64[c]	0.76[c]	0.087[bc]	0.305[b]	4.43[c]
SEM	-	0.13	0.03	0.04	0.003	0.02	1.54

[a-c] Means within a column with no common superscripts differ significantly ($p<0.05$).

* Organ weight toxicity index (OWTI) = weight of liver×heart×kidney×spleen×pancrease×100.

Table 5. Effects of dietary FERMKIT and aflatoxin on duckling hematology values and serum biochemical values (28 days of age) (Experiment 1)

Dietary treatment		Hematology values			Serum biochemical values				
Aflatoxin ppb	FERMKIT %	Hb, g/dl	HCT, %	HTI*	Protein (mg/dl)	Glucose (mg/dl)	Ca (mg/dl)	P (mg/dl)	SBVTI**
<10	-	9.20[a]	41[a]	377.4[a]	4.90[a]	235[a]	13.50[a]	6.60[a]	102.55[a]
30	-	8.80[ab]	36[b]	317.0[bc]	4.45[ab]	200[bc]	13.30[a]	6.15[a]	72.95[b]
60	-	8.30[bc]	33[bc]	274.0[cd]	4.30[ab]	188[c]	12.35[b]	5.30[b]	52.70[c]
120	-	8.00[c]	29[c]	231.9[d]	3.90[b]	168[d]	12.05[b]	4.40[c]	34.68[d]
120	0.30	8.30[bc]	34[b]	282.3[bc]	4.65[a]	200[bc]	12.25[b]	6.50[a]	74.27[a]
120	0.60	8.90[a]	37[ab]	329.1[b]	4.90[a]	214[b]	13.80[a]	6.55[a]	94.72[a]
SEM	-	0.20	1.73	18.82	0.24	6.24	0.36	0.29	5.61

[a-d] Means within a column with no common superscripts differ significantly ($p<0.05$).

* Hematology toxicity index (HTI) = Hb×HCT.

** Serum biochemical value toxicity index (SBVTI) = protein×glucose×Ca×P 10^{-3}.

0.05% and 33% for the 0.10% inclusion levels. Data in Table 5 showed that feeding diet contaminated with AF at the level 120 ppb significantly reduced ($p<0.05$) blood hemoglobin, hematocrit and serum biochemical values (glucose, protein, Ca and P content). Similar results have also been reported by several researchers (Mohiuddin et al., 1986; Kubena et al., 1990a, b; Khajarern et al., 1990; Huff et al., 1992; Schell et al., 1993). Adding FERMKIT at 0.6% of the 120 ppb AF diet significantly ($p<0.05$) diminished the adverse effects of AF on all of the above parameters. In addition, Cureo et al. (1988) used chitosan or derivative of chitin to control growth of AF producing fungi and noted that chitin-chitosan could successfully inhibit fungal growth and AF production.

Data in Table 6 present a method for assessment of toxin binder efficacy, toxin absorbance index (TAI). The TAI was calculated by multiplying hematology toxicity index (HTI) by serum biochemistry values toxicity index (SBVTI) and economic loss index (ELI). The resulting TAI clearly demonstrated that addicting 0.6% FERMKIT to the 120 ppb AF duckling diets significantly diminished ($p<0.05$) the adverse effect of AF toxicity to a degree nearly comparable to the uncontaminated diet. TAI and relative absorbance index (RAI) are sensitive indicators for assessing the efficacy of a sequestrant (toxin binder). In case of FERMKIT, addition at 0.3% of the 120 ppb AF diets showed the sequestering efficacy for AF at 52.70%, whereas that at 0.6% inclusion was 79.85% of AF.

Table 6. Effects toxin absorbance index (TAI) by using hematology toxicity index (HTI), serum biochemical value toxicity index (SBVTI) and economic loss index (Experiment 1)

Dietary treatment		HTI	SBVTI	ELI	Feather score (FC)	Toxin absorbance index (TAI)*	Relative absorbance index (RAI)%**
Aflatoxin ppb	FERMKIT %						
<10	-	377.4[a]	102.55[a]	898[a]	2.54[a]	34.735[c]	100
30	-	317.0[bc]	72.95[b]	858[ab]	2.16[b]	19.905[c]	-
60	-	274.0[cd]	52.70[c]	796[b]	1.78[cd]	11.525[d]	-
120	-	231.9[d]	34.68[d]	464[c]	1.55[d]	3.730[e]	-
120	0.30	282.3[bc]	74.27[b]	876[ab]	1.96[bc]	18.305[c]	52.70
120	0.60	329.1[b]	94.72[a]	890[a]	2.20[b]	27.735[b]	79.85
SEM	-	18.82	5.61	31.79	0.12	2.07	-

[a-e] Means within a column with no common superscripts differ significantly ($P<0.05$).

* Toxin absorbance index = HTI×SBVTI×ELI×FC×10^{-6}.

** Relative toxin absorbance index (RAI) = 100×TAI (with FERMKIT)/TAI (<10 ppb AF).

Table 7. Effects of dietary FERMKIT and zearalenone (ZEN) on duckling weight gain, feed efficiency, percentage of mortality and livability and economic loss index (ELI) (Experiment 2)

Dietary treatment		Feed intake, kg	Mean body weight gain, g	Feed conversion (g feed :g gain)	Mortality %	Livability %	Economic loss index*
Zearalenone ppb	FERMKIT %						
<10	-	2.09	892[a]	2.35[b]	4.00	96	856[a]
150	-	1.99	658[b]	3.02[a]	12.00	88	579[b]
150	0.60	1.74	711[b]	2.44[b]	2.00	98	696[b]
SEM	-	0.13	22.13	0.10	4.90	4.90	43.38

[a-b] Means within a column with no common superscripts differ significantly ($p<0.05$).

* Economic loss index (ELI) = mean body weight gain×livability×10^{-2}.

Table 8. Effects of dietary FERMKIT on duckling organ weights of oviducts, testis, burza of fabricius and cloaca eversion score at 4 weeks of age (Experiment 2)

Dietary treatment		Organ weight, g/kg BW			Cloaca eversion score[1]	Organ weight toxicity index (OWTI)*
Zearalenone ppb	FERMKIT %	Bursa of fabricius	Testis	Oviduct		
<10	-	0.867[a]	0.099[a]	0.137[b]	0.06[b]	855[a]
150	-	0.674[b]	0.068[b]	0.184[a]	0.60[a]	456[b]
150	0.60	0.789[ab]	0.089[a]	0.174[a]	0.18[b]	703[a]
SEM	-	0.05	0.004	0.01	0.04	72.62

[a-b] Means within a column with no common superscripts differ significantly ($p<0.05$).

[1] Cloaca eversion score: Range of 0 to 3, 0 = normal, 1 = slightly prolapse, 2 = moderate prolapse and 3 = severely prolapse.

* Organ weight toxicity index (OWTI) = bursa of fabricius×testis×10^{4} .

Table 9. Evaluation toxin absorbance index (TAI) by using organ weight toxicity index (OWTI) and economic loss (ELI) (Experiment 2)

Dietary treatment		OWTI	ELI	Toxin absorbance index(TAI)*	Relative absorbance index(RAI), %**
Zearalenone ppb	FERMKIT %				
<10	-	855[a]	856[a]	729.20[a]	100.00
150	-	456[b]	579[b]	265.84[c]	-
150	0.60	703[a]	696[b]	489.38[b]	67.11
SEM	-	72.62	43.38	50.50	-

[a-c] Means within a column with no common superscripts differ significantly ($p<0.05$).

* Toxin Absorbance Index (TAI)=OWTI×ELT×10^{3}.

** Toxin Absorbance Relative (RAI)=100×TAI (with FERMKIT), TAI (<10 ppm ZEN).

Experiment 2

Data in Table 7 summarized the toxological effects of ZEN, at 150 ppb of diet, on performance of ducklings in terms of feed intake, mean body weight gain, feed conversion, mortality, livability and economic loss index (ELI) which calculated from mean body weight gain and livability. Ingestion of ZEN, at 150 ppb of diet caused significant ($p<0.05$) decreases in mean body weight gain, ELI, and poorer feed efficiency. Feed intake and livability were also decreased but were not statistically significant ($p>0.05$). Mortality rate ranged from 4% for the control to 12% for the 150 ppb ZEN treatment. The overall weight gain of the 150 ppb ZEN ducklings was reduced by 26.23%, whereas feed efficiency was lowered by 28.82% when compared with the controls. Addition of FERMKIT, at 0.6% of the ZEN contaminated diet, diminished those adverse effects to an appreciable degree; however, the mean body weight and ELI were not fully restored to the levels of the control. This might be caused by a slightly lower feed intake of the ZEN ducklings than that of the uncontaminated one. Data in Table 8 showed the effect of ZEN on development of some selected organs and the ameliorating effects of FERMKIT against the ZEN toxicity. The relative weights of Bursa of Fabricius, testicles, oviducts and cloaca eversion score were significantly affected ($p<0.05$) by feeding diet contaminated with 150 ppb ZEN. The ZEN, at 150 ppb of diet, caused decreases in weights of testicles and Bursa of Fabricius of the ducks when continuously ingested throughout the period of 1-28 days of age. These results support previous findings by Chi et al. (1980a, b) and Allen et al. (1981a, b) which indicated that purified ZEN might accelerate oviduct development in female and retard testicles growth in young male chickens. Addition of 0.6% FERMKIT to diet containing 150 ppb ZEN significantly diminished ($p<0.05$) the adversely affected development of testicles, oviducts, Bursa of Fabricius and cloaca eversion score. The OWTI of the FERMKIT ducklings was restored to a statistically comparable level of the control.

Table 9 showed the assessment of the toxin absorbance index (TAI) of FERMKIT for ZEN, calculated by

multiplying the organ weight toxicity index (OWTI) by the economic loss index (ELI). The TAI clearly indicated that the added FERMKIT, at 0.6% of the 150 ppb ZEN diet, significantly diminished the adverse effects of ZEN toxicity in the young ducks from 1-28 days of age and showed sequestering efficacy for ZEN at 67.11%.

ACKNOWLEDGEMENT

The author gratefully acknowledge Easy-Bio System, Inc., Korea and Paiboon Watana Co., Ltd., Thailand for their excellent technical assistance and gracious financial support for this study.

REFERENCES

Allen, N. K., C. J. Mirocha, S. Aakhus-Allen, J. J. Bitgood, G. Weaver and F. Bates. 1981a. Effect of dietary zearalenone on reproduction of chickens. Poult. Sci. 60:1165-1174.

Allen, N. K., C. J. Mirocha, G. Weaver, S. Aakhus-Allen and F. Bates. 1981b. Effect of dietary zearalenone on finishing broiler chickens and growing turkey poults. Poult. Sci. 60: 124-131

AOAC. 1995. Official Methods of Analysis. Association of Official Analytical Chemists. Washington, D.C., p.1141

Applebaum, R. S. and E. H. Marth. 1982. Use of sulphite of bentonite to eliminate aflatoxin M_1 from naturally contaminated raw whole milk. Z. Lebensm. Unters. Forsch. 174:303-305.

Bacon, C. W. and H. L. Marks. 1976. Growth of broilers and quail fed Fusarium (Giberella zeae) infected corn and zearalenone. Poult. Sci. 55:1531-1535.

Baird, J. D. 1978. Study on the toxicosis of *Aspergillus flavus* and *A. paraciticus* in domestic and laboratory animals with particular reference to the duckling and the sheep. Ph.D. Thesis, University of Sydney. Australia.

Bryden. W. L. 1982. Aflatoxins and animal product: An Australian Perspective. Food Technol. Aust. 34:216-223

Bursian. S. J., R. J. Aulerich, J. K. Cameron, N. K. Ames and B. A Steficek. 1992. Efficacy of hydrated sodium calcium aluminosilicate in reducing the toxicity of dietary zearalenone to mink. J. Appl. Toxicol. 12:85-90.

CAST. 1989. Mycotoxins: Economic and health risk. Task Force Rep. No. 116, November 1989. Council of Agriculture Science and Technology. Ames, IA.

Chi, M. S., C. J. Mirocha, H. J. Kurtz, G. A. Weaver, F. Bates, T. Tobison and W. Shimoda. 1980a. Effect of dietary zearalenone on growing broiler chicks. Poult. Sci. 59:531-536.

Chi, M. S., C. J. Mirocha, G. A. Weaver and H. J. Kurtz. 1980b. The acute effect of zearalenone on the White Legnorn female. Appl. Environ. Microbiol. 39:1026-1030.

Chung, T. K., J. W. Erdman and D. H. Baker. 1990. Hydrated sodium calcium aluminosilicate : effects on zinc, manganese, vitamin A and riboflavin utilization. Poult. Sci. 69:1364-1370.

Cuero, T. G., E. B. Lillehoj, T. E. Cleveland and A. H. Reine. 1988. Chitosan as a control agent of toxicogenic fungal growth and aflatoxin production. Proc. Jpn. Assoc. Mycotoxicol. (suppl.): 1: 194-198.

Dailey, R. E., R. E. Reese and E. A. Brouwer. 1980. Metabolism of (14C) zearalenone in laying hens. J. Agric. Food Chem. 28: 286-291.

Danicke, S. 2001. Prevention and control of mycotoxins in the poultry production chain. 13th Symp. Poult. Nutr. Oct. 2001, Blankenberge, Belgium.

Davidson, J. N., J. G. Babish, K. A. Delaney, D. R. Taylor and T. D. Phillips. 1987. Hydrated sodium calcium aluminosilicate decreases the bioavailability of alfatoxin in the chicken. Poult. Sci. 66(Suppl. 1):89.

Dicken, J. W., W. F. McClure and T. B. WhiteTaker. 1980. Densitometric equipment for rapid quantitative thin-layer chromatograph of aflatoxin. J. Am. Oil. Chem. Soc. 57:205-208.

Duncan, D. D. 1955. Multiple range and multiple F tests. Biometrics. 11:1-42.

Ellis, J. A., R. B. Harvey, L. F. Kubena, R. H. Bailey, B. A., Clement and T.D. Phillips. 1990. Reduction of aflatoxin M1 residues in milk utilizing hydrated sodium calcium aluminosilicate. Toxicologist 10(1):163.

Ellis, J. A., R. H. Bailey, B. A., Clement and T. D. Phillips. 1991. Chemisorption of aflatoxin M1 from milk by hydrated sodium calcium aluminosilicate. Toxicologist 11:96.

Harvey, R. B., L. F. Kubena, T. D. Phillips, W. E., Huff and D. E. Corrier. 1989. Prevention of clinical signs of aflatoxicosis with hydrated sodium calcium aluminosilicate added to diets. Proc. 20th Annu. Meet. Am. Assoc. Swine Pract. pp.99-102.

Harvey, R. B., L. F. Kubena, M. H. Elissalde and T. D. Phillips. 1993. Efficacy of zeolitic ore compounds on the toxicity of aflatoxin in growing broiler chickens. Avian Dis. 37:67-73.

Huff, W. E., E. F. Kubena, R. B. Harvey and T. D. Phillips. 1992. Efficacy of hydrated sodium calcium aluminosilicate to reduce the individual and combined toxicity of aflatoxin and ochartoxin A. Poult. Sci. 71:64-69.

Khajarern, J., S. Khajarern and C. Ratanasethakul. 1990. Efficacy of hydrated sodium calcium aluminosilicate (Novasil) on bioavailability of aflatoxin in the ducks. Proc. 7th FAVA Congress. Pattaya. Thailand. pp.381-390.

Khajarern, J., S. Khajarern and C. Ratanasethakul. P. Phundee, V. Chaikan, A. Srisataporn, P. Sakiya. S. Chaiput, P. Sripraya, B. Thummabutr, D. Suprasert, M. Bunyaratpalin, A. Chaimongkul, K. Supamac and C. Burisut. 2001. Safety of animal feeds produced from peanuts and corn in the aflatoxin controlled system. Annual Report 2000. Dept. Anim. Sci., Khon Kaen University, Khon Kaen, Thailand. Krogh, P. 1976. Epidemiology of mycotoxic porcine nephropathy. Nord. Vet. Med. 28:452-458.

Kubena. L. F., R. B. Harvey, W. E. Huff, D. E. Corrier, T. D. Phillips and G. E. Rottinghaus. 1990a. Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. Poult. Sci. 69:1078-1086.

Kubena. L. F., R. B. Harvey, T. D. Phillips and B. A. Clement. 1993. Effect of hydrated sodium calcium aluminosilicate on aflatoxicosis in broiler chicks. Poult. Sci. 72:651-657.

Kubena, L. F., R. B. Harvey, T. D. Phillips, D. E. Corrier and W. E. Huff. 1990b. Diminution of aflatoxicosis in growing chickens by the dietary addition of a hydrated sodium calcium aluminosilicate. Poult. Sci. 69:727-735.

Kubena, L. F., W. E. Huff, R. B. Harvey, A. G. Yersin, M.H.

Elissalde, D. A. Witzel, L. E. Giroir, T. D. Phillips and H.D. Peterson. 1991. Effects of a hydrated sodium calcium aluminosilicate on growing turkey poults during aflatoxicosis. Poult. Sci. 70:1823-1830.

Kurtz, H. J. and C. J. Mirocha. 1978. Zearalenone (F_2) induced estrogenic syndrome in swine. In: Mycotoxic fungi, Mycotoxins, Mycotoxicoses. Vol. 2. (Ed. T.D. Wyllie and L.G. Morchouse). Marcel Dekker, New York. pp. 1256-1264.

Maryamma, K. I., C. B. Manomoham, M. G. Nair, P. K. Ismail, T. Streekumatan and A. Rajan. 1992. Pathology of zearalenone toxicosis in chicken and evaluation of zearalenone residues in tissues. Indian J. Anim. Sci. 62:105-107.

Mirocha, C. J., C. M. Christensen and G. H. Nelson. 1971. F-2 (zearalenone) estrogenic mycotoxin from Fusarium. In: Microbial toxins. Vol 7 (Ed. S.J. Ajl, S.Kadis and A Ciegler). Academic Press, New York, NY. pp.107-138.

Mirocha, C. J., S. V. Pathre and C. M. Christensen. 1977. Zearalenone. In: Mycotoxins in human and clinical health. (Ed. J.V.Rodricks, C.W. Hesseltine and H.A. Mehlman). Pathotox. Publ. Inc., Park Forest, South Il. U.S.A.

Mirocha, C. J., T. S. Robinson T. J. Pawlosky and N. K. Allen. 1982. Distribution and residue determination of (3H) zearalenone in broilers. Toxicol. Appl. Pharm. 66:77-78.

Mohiuddin, S. M., M. V. Reddy, M. M. Reddy and K. Ramakrishna. 1986. Studies on phagocytic activity and hematological change in aflatoxicosis in poultry. Indian Vet. J. 63:442-445.

Palmgren, M. S. and A. W. Hayes. 1987. Aflatoxin in food. In: Mycotoxins in food. (Ed. P. Krogh). Academic Press, New York. pp.65-95.

Phillips, T. D., L. F.Kubena, R. B. Harvey, D. R. Taylor and N. D. Heidelbaugh. 1987. Mycotoxin hazards in agriculture: new approach to control. J. Am. Vet. Med. Assoc. 12: 1617.

Phillips, T. D., L.F.Kubena, R. B. Harvey, D. R. Taylor and N. D. Heidelbaugh. 1988. Hydrated sodium calcium aluminosilicate : a high affinity sorbent for aflatoxin. Poult. Sci. 67:243-247.

Phillips, T. D., A. B. Sarr, B. A. Clement, L. F. Kubena and R. B. Harvey. 1990. Prevention of aflatoxicosis in farm animals via selective chemisorption of aflatoxin. In: Mycotoxins, cancer and health.

Pennington Center Nutrition Series vol l, Louisiana State University Press, Baton Rouge, Lousiana, U.S.A. pp.223-227.

Phillips, T. D., B. A.Clement and D. L. Park. 1994. Approaches to reduction of aflatoxins in foods and feeds. In: The Toxicology of Aflatoxins : Human Health, Veterinary and Agricultural Significance. (Ed. D.L. Eaton and J.D. Groopman). Academic Press, New York, New York. pp.383-389.

Ramos, A. J. 1994. Prevencion de los efectos cancerigenos de las aflatoxinasy la zearalenona mediante el empleo de compuestos adsorbents no nutritivos. Estudios *in vitro*. Ph. D. Thesis. Universidad Politecnica de Valencia, Spain.

Ramos, A. J., J. Fink-Gremmels and E. Hernandez. 1996. Prevention of toxic effects of mycotoxins by means of nonnutritive absorbent compounds. J. Food Protect. 59:631-641.

Richard, J. L., J. R. Thurston and A. C. Pier. 1978. Effects of mycotoxin on immunity. In: Toxins : Animal, plant and microbial. (Ed. P. Rosenberg). Permagon Press, New York. pp. 801-817.

SAS Institute Inc. 1989. SAS/STAT User's Guide : Version 6,4th ed., Vol 2 SAS Institute Inc., Cary, N. C. Schell, T. C., M. D. Lindeman, E. T. Kornegay, D. J. Blodgett and J. A. Doerr. 1993. Effectiveness of different types of clay for reducing the detrimental effects of aflatoxin-contaminated diets on performance and serum profiles of weaning pigs. J.Anim. Sci. 71:1226-1231.

Shotwell, D, L., C. W. Hesseltine, R. D. Stubblefield and W. G. Sorenson. 1996. Production of aflatoxin on rice. Appl. Microbiol. 14:425-428.

Suttajit, M. 1989. Prevention and control of mycotoxins. Thai. J. Toxicol. 5:17.25.

Teng, B. K. G. 1974. The Chemistry of Clay-Organic Reactions. Adam Hilger, London. West. S. R. D. Wyatt and P.B. Hamilton. 1973. Increased yield of alfatoxin by incremental increases of temperature. Appl. Microbiol. 25:1018-1019.

Wogan, G. N. and W. F. Busby. Jr. 1980. Toxic Constituents of Plant Foodstuff. 2rd Ed. Academic Press, New York, New York.

Wood, G. E. 1992. Mycotoxin in foods and feeds in the United States. J. Anim. Sci. 70:3941-3949.

WHO. 1989. Prevention of liver cancer. WHO Technical Report Series, Vol. 691. World Health Organization, Geneva, Switzerland.

Wyllie, T. D. and L. G. Morehouse. 1977. Mycotoxic fungi, Mycotoxins, Mycotoxicosis Vol. 2. Marcel Dekker, New York, NY.