EFFECTS OF VITAMIN E AND SELENIUM SUPPLEMENTATION TO DIETS CONTAINING AFLATOXIN B_1 ON THE CONTENTS OF LIVER LIPIDS AND VARIOUS BLOOD PARAMETERS IN RATS

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Summary

Ninety Wistar male rats were used to study the effects of vitamin E and Se supplementation to diets containing aflatoxin B_1 on the contents of liver lipids and various blood parameters. Two levels of dietary aflatoxin (0 and 1 ppm), 3 levels of vitamin E (30, 60 and 120 IU/kg), and 3 levels of Se (0.1, 1 and 2 ppm) were used to design a $2 \times 3 \times 3$ factorial experiment. Rats, weighing about 200 g, were randomly allotted to 18 cages, 5 rats per cage. The aflatoxin significantly ($p < .05$) decreased growth rate, feed intake and feed efficiency. Aflatoxin increased the glucose level and decreased the cholesterol level in blood significantly. Levels of blood triglyceride, total protein, and albumin were not affected by aflatoxin, vitamin E or Se. Activities of blood alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly increased by aflatoxin; however, the glutathione peroxidase (GSH-Px) activity in the blood was decreased by aflatoxin even in the presence of Se. The vitamin E supplementation decreased the AST activity significantly, while GSH-Px activity increased significantly as the levels of dietary Se increased. The levels of total cholesterol and free cholesterol in the liver were significantly lower in rats receiving aflatoxin, while the extra vitamin E supplementation increased these hepatic cholesterol levels. It was concluded that the extra dietary vitamin E or Se supplementation might partially alleviate some of the harmful effects of aflatoxin in rats.

(**Key Words** : Rats, Vitamin E, Se, Aflatoxin, Glutathione Peroxidase)

Introduction

Aflatoxin, metabolite produced by certain strains of *Aspergillus flavus* in poorly stored feeds such as corn and peanut oil meal, is a group of highly toxic agents for many animal species. Metabolic cascade of aflatoxin is occurred by binding aflatoxin to reactive epoxide at the 2, 3 position of the terminal furan ring, and subsequently produced electrophilic epoxide has been believed to play a major role in aflatoxicosis including malfunction of protein synthesis, loss of enzyme activities, and carcinogenesis (Gurtoo and Dave, 1975; Swenson et al., 1977; Lin et al., 1978). The other harmful effects of aflatoxin are associated with alteration of lipid metabolism, imbalance of immune system, depression of liver function, retardation of growth, and subsequent death. Although there are more than 15 known aflatoxin compounds, aflatoxin B_1 (Asao et al., 1965) is the most harmful.

Several studies have shown the beneficial effects of sulfur-containing amino acids, glutathione peroxidase (GSH-Px), and antioxidants in preventing aflatoxicosis. According to these studies, vitamin E was highly effective in preventing free radical formation (Combs et al., 1974). In addition, Se was found to play its antioxygenic role as a component of GSH-Px (Rotruck et al., 1973). Numerous *in vitro* studies have demonstrated the preventive effect of dietary supplementation of Se or vitamin E against the biological membrane peroxidation.

Degan and Neumann (1978) reported that high concentration of aflatoxin B_1-GSH-Px conjugate, a non toxic metabolite, was produced in bile acid from rats fed diets containing aflatoxin with Se. Thus, dietary addition of Se may alleviate the aflatoxicosis by increasing GSH-Px activity and eventual changes of toxic substances to

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inert metabolites. Todd et al. (1968) reported that the supplementation of chlorotetracycline and vitamin A, E, and K to diets containing 8 ppm of aflatoxin increased the body weight of rats, even if the clinical signs, mortality and histopathological changes were not affected. The objectives of the present study were to evaluate the effects of dietary addition of vitamin E and Se on animal performance, GSH-Px activity, lipid composition of the liver and blood biochemical profiles when rats were fed diets containing aflatoxin.

Materials and Methods

Ninety Wistar male rats (7-wk-old) with an average weight of 200 g were randomly allotted to 18 cages, 5 rats per cage, in a 2 × 3 × 3 factorial with two levels of aflatoxin (0 and 1 ppm), three levels of vitamin E (30, 60 and 120 IU/kg), and three levels of Se (0.1, 1.0 and 2.0 ppm). Feed and water were supplied *ad libitum*. The experimental period consisted of 7 days for adjustment and 21 days for a feeding trial. The animal room was maintained at 21℃, 60% humidity, and 12 L : 12 D in illumination.

The composition of the basal diets are presented in table 1. The vitamin E (Merck Darmstadt, α-tocopherol) and aflatoxin B_1 (Sigma) dissolved into dimethyl sulfate were added to the corn-soybean meal basal diet according to the experimental design. Feed intake and individual body weight were recorded weekly. At the end of the feeding trial, all animals were anesthezied with ether and the blood collected by heart puncture was placed in a bottle containing Na-EDTA. Plasma samples were harvested by 10 min centrifugation at 3,000 rpm and kept at −70℃ for later use. Immediately after collection of blood from rats, the liver, spleen, kidney, and adrenal gland were separated and weighed. The liver tissues were frozen at −70℃ to measure the level of cholesterol and GSH-Px activity.

A clinical chemical auto analyzer (Gilford Co. Impact 400 E) was employed for the determination of total protein, total albumin, blood cholesterol, triglyceride, total glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and globulin in serum. The activity of blood GSH-Px was measured with a spectrophotometer at 340 nm using a Gilford-diagnostic kit. Aflatoxin analysis of the liver consisted of extraction of the toxins by AOAC (1984) and application to HPLC (Kontron HPLC System 600, Switzerland). The liver cholesterol was measured colorimeterically at 560 nm. The cholesteryl ester was obtained by subtracting free cholesterol from total cholesterol. Total lipid in the liver extracted by 2:1 (v/v) chloroform:methanol (Folch et al., 1957) was determined by weight differences.

Data were analyzed by 2 × 3 × 3 factorial using Proc GLM in SAS (1985) according to Snedecor and Cochran (1967). When main effects were found significant, LSD test was conducted to compare the mean differences. The limit of probability accepted as being significant was $p < 0.05$.

TABLE 1. COMPOSITION OF THE BASAL DIET[1]

Ingredients	Percentage
Yellow corn	40.00
Wheat flour	3.90
Wheat bran	5.00
Wheat germ meal	5.00
Fish meal	2.00
Soybean meal	28.50
Corn gluten meal	3.90
Limestone	0.91
Tapioca	2.44
Salt	1.00
Fat	3.60
Brewers yeast	2.00
Choline	0.04
DL-Methionine (50%)	0.51
Vit.-min. premix[2]	1.00
Pellet binder	0.10
Total	100.00

[1]Contained 12.8% moisture, 27.53% crude protein, 5.59% crude fat, 4.14% crude fiber, and 9.5% ash.

[2]Contained per kg of the mixture: $MnSO_4H_2O$, 1.0 g; $ZnCl_2$, 2.0 g; $FeSO_47H_2O$, 8.0 g; $CuSO_45H_2O$, 1.0 g; KI, 0.03 g; $CoSO_4$, 0.1 g; $MgSO_4$, 100.0 g; Na_2SO_4, 1.5 g; vitamin A, 3,000,000 IU; vitamin D_3, 600,000 IU; vitamin E-acetate, 6.0 g; vitamin K_3, 6.0 g; thiamine-HCl, 1.2 g; riboflavin, 1.6 g; pyridoxine-HCl, 1.6 g; cyanocobalamine, 1.2 g; Ca-D-pantothenate, 2.9 g; choline chloride, 250.0 g; niacin, 4.0 g; inositol, 8.0 g; biotin, 0.04 g.

Results and Discussion

Weight gain, feed intake, and feed efficiency

The effects of aflatoxin, vitamin E or Se on body weight, feed intake, and feed efficiency are presented in table 2. The growth rate was significantly decreased by the dietary addition of aflatoxin. The supplementation of vitamin E and Se did not show any effect in this respect. There were interactions in aflatoxin × vitamin E and vitamin E × Se. The dietary addition of vitamin E and Se

did not induce any consistent tendency in animal growth, feed intake, and feed efficiency.

TABLE 2. EFFECTS OF DIETARY AFLATOXIN, VITAMIN E, AND SE ON BW GAIN, FEED INTAKE, AND FEED EFFICIENCY OF MALE RATS (N = 5)

Treatments			BW gain	Feed intake	Feed efficiency
Aflatoxin	Vitamin E	Se			
mg/kg	IU/kg	mg/kg	····· g/d ·····		
0	30	0.1	4.37	25.4	5.62
		1.0	4.85	27.6	5.74
		2.0	4.51	26.9	6.05
	60	0.1	4.75	26.9	5.76
		1.0	4.49	27.1	6.17
		2.0	4.55	25.5	5.71
	120	0.1	3.70	26.6	7.27
		1.0	3.85	26.1	6.82
		2.0	4.89	27.2	5.56
1	30	0.1	3.22	25.6	8.08
		1.0	3.65	26.1	7.17
		2.0	3.11	24.1	7.77
	60	0.1	3.56	26.6	7.52
		1.0	2.65	25.0	8.88
		2.0	2.88	24.2	8.49
	120	0.1	3.26	25.0	7.75
		1.0	3.77	27.0	7.23
		2.0	3.99	26.5	6.63
LSD (.05)			0.60	2.65	1.13
Main effects					
Aflatoxin (AF)			*	*	*
Vitamin E (VE)			NS	NS	NS
Se			NS	NS	NS
AF × VE			*	NS	*
AF × Se			NS	NS	NS
VE × Se			*	NS	*
AF × VE × Se			NS	NS	NS

*$p < .05$.

The feed intake and feed efficiency in rats fed aflatoxin diets were remarkably decreased as compared with those of rats fed aflatoxin-free diets, however, the effects of vitamin E and Se on feed intake in both aflatoxin and aflatoxin-free diets were not noticeable. This decreased feed efficiency may be due to disturbed secretion of pancreatic digestive enzymes in aflatoxicosis (Osborne and Hamilton, 1981). Significant interactions ($p < .05$) among groups of the rats fed vitamin E, aflatoxin, and Se were observed. These results agreed with other reports that supplementation of 5 ppm (Hamilton et al., 1974) and 2.5 ppm (Pearson et al., 1990) aflatoxin to poultry diets resulted in a significant decrease in body weight. Aflatoxin × vitamin E interaction is the most interesting one. Apparently, feeding aflatoxin reduced BW gain, feed intake, and feed efficiency and supplementation of vitamin E and Se did not exert any effect on these parameters in rats.

Biochemical profiles of blood composition

The effects of dietary addition of aflatoxin, vitamin E and Se on the biochemical profiles of blood are presented in table 3. Feeding aflatoxin increased the blood glucose significantly, however, the supplementation of vitamin E decreased the level of it. The addition of Se to aflatoxin-free diets significantly reduced blood glucose, but the content of blood glucose was not affected by addition of Se to aflatoxin diet. The total blood cholesterol was markedly decreased in rats received aflatoxin compared to controls. Vitamin E and Se did not affect the level of blood cholesterol. Vitamin E did not cause any fluctuation in blood triglyceride concentration in rats given aflatoxin-free diets, but the supplementation of only 30 IU vitamin E/kg to aflatoxin diet tended to increase the blood triglyceride. The total blood protein was not directly influenced by any dietary treatment. The blood albumin contents were affected by added Se. The supplementation of Se to aflatoxin-free diet gave rise to the increase in blood albumin. The values of blood globulin were tended to be increased by the both aflatoxin and aflatoxin-free diets which are fortified with vitamin E.

Our result regarding the increased blood glucose in response to dietary aflatoxin is not in agreement with reports of Manning et al. (1991a, b), but their results indicating decreased blood cholesterol corresponded to our data. The fact that feeding aflatoxin did not change the blood total protein and albumin in our experiment does not agree with results of Kubena et al. (1990) and Manning et al. (1990b) which showed decreases in total protein and albumin. In addition, the previous report (Jack Yang et al., 1976) that vitamin E reduced the blood total protein and cholesterol was quite different from those of our observations. Above all, this experiment indicated that blood cholesterol was significantly decreased in response

TABLE 3. EFFECTS OF DIETARY AFLATOXIN, VITAMIN E, AND SE ON BLOOD BIOCHEMICAL PROFILES AND ENZYME ACTIVITIES OF MALE RATS (N = 5)

Treatments									Enzyme activities		
Aflatoxin	Vit E	Se	Glucose	Cholesterol	Triglyceride	Total protein	Albumin	Globulin	ALT[1]	AST[2]	GSH-Px[3]
mg/kg	IU/kg	mg/kg	····· mg/dL ·····			g/dL	U/L	g/dL	··· U/L ···		EU/g, Hb
0	30	0.1	177	79.0	146	5.98	3.94	2.02	43.2	181	161
		1.0	155	75.2	139	6.12	4.04	2.12	44.4	127	194
		2.0	137	74.6	122	6.22	4.16	2.12	43.2	143	216
	60	0.1	154	72.2	133	5.94	3.92	1.96	38.4	161	167
		1.0	141	88.0	137	6.26	4.16	2.12	34.0	105	202
		2.0	132	82.6	141	6.36	4.24	2.12	35.4	120	241
	120	0.1	126	72.6	138	6.24	4.14	2.18	41.8	172	187
		1.0	128	79.4	144	6.34	4.18	2.16	47.0	189	241
		2.0	131	76.4	133	6.34	4.20	2.16	44.6	183	273
1	30	0.1	137	68.8	125	6.38	4.18	2.06	52.4	195	103
		1.0	156	70.2	127	6.30	4.22	2.12	56.2	188	115
		2.0	156	75.0	127	6.24	4.14	2.04	52.2	186	135
	60	0.1	153	76.2	136	6.34	4.18	2.18	52.2	199	136
		1.0	156	69.2	148	6.06	3.98	2.06	52.8	191	135
		2.0	152	76.2	150	6.20	4.16	2.10	51.4	186	129
	120	0.1	151	76.8	148	6.36	4.12	2.24	54.0	199	115
		1.0	141	72.0	144	6.20	4.10	2.14	50.4	191	134
		2.0	141	72.8	149	6.22	4.12	2.10	51.8	186	153
LSD (.05)			17.5	6.57	18.3	0.25	0.16	0.15	5.12	19.0	30.3
Main effects											
Aflatoxin (AF)			*	*	NS	NS	NS	NS	*	*	*
Vitamin E (VE)			*	NS	*	NS	NS	*	*	*	*
Se			NS	NS	NS	NS	*	NS	NS	*	*
AF × VE			*	NS	*	NS	*	NS	*	*	*
AF × Se			*	*	NS	*	*	*	NS	*	*
VE × Se			NS	NS	NS	NS	NS	NS	NS	*	*
AF × VE × Se			*	*	NS	NS	NS	NS	NS	*	*

*$p < .05$.
[1]Alanine aminotransferase.
[2]Aspartate aminotransferase.
[3]Glutathione peroxidase.

to the dietary addition of aflatoxin.

Blood ALT, AST and GSH-Px activities

Data on the activities of blood ALT, AST, and GSH-Px are presented in table 3. The blood ALT and AST activities were significantly increased by aflatoxin. Significant effects of 60 IU vitamin E/kg supplementation on these enzyme activities in rats fed aflatoxin-free diets were observed, however, the addition of vitamin E to diets containing aflatoxin did not affect ALT activity. The

dietary addition of 60 IU vitamin E/kg to aflatoxin diet markedly decreased blood AST activity and 120 IU vitamin E/kg to the aflatoxin diet significantly increased the blood AST activity. Generally, about 30 to 40 IU/kg of vitamin E is recommended for rat diets (NRC, 1978). The increased ALT activity by the feeding of aflatoxin is in accordance with that of Dalvi and McGowan (1984), who conducted a study to evaluate the effect of aflatoxin on broilers. Also, the results which indicate the increased blood AST activity in response to aflatoxin diet supplemented with 120 IU vitamin E/kg are similar with the result from Ewan and Wastell (1970).

The activity of GSH-Px was significantly decreased in response to feeding aflatoxin. Vitamin E increased the GSH-Px activity only in the rats fed aflatoxin-free diets. Se increased GSH-Px activity in the liver of the rats received diets containing either aflatoxin or no-aflatoxin. Chen et al. (1982) noted that aflatoxin diets supplemented with Se markedly increased the blood GSH-Px activity. They also reported that the vitamin E reduced the formation of aflatoxin B_1 adducts in the liver. Weiss (1990) also reported that GSH-Px activity was increased by the dietary Se. The observation that the rats fed aflatoxin-free diet containing 120 IU vitamin E/kg showed significantly enhanced blood GSH-Px is in accordance with other reports in pigs (Rasmussen, 1974) and poultry (Combs, 1978). Jack Yang et al. (1976) mentioned that blood GSH-Px activity was decreased in the animals fed excessive (10 fold greater than requirement) or very small amount of vitamin E. The average requirement of vitamin E in rats is 30 to 40 IU/kg (NRC, 1978).

In conclusion, feeding aflatoxin decreased the activity of GSH-Px and the toxicity resulting from feeding aflatoxin could be alleviated by the supplementation of Se.

Total lipid and cholesterol contents in the liver

The effect of aflatoxin, vitamin E and Se on lipid and cholesterol contents of the liver are presented in table 4. The feeding of aflatoxin diets containing 30 IU vitamin E/kg induced a significant increase in the liver lipid contents, but higher levels of vitamin E nullified this effect of aflatoxin. When rats were fed aflatoxin-free diets, the lipid contents were elevated as the levels of vitamin E increased.

Results of the liver lipid contents from the present experiment were similar to the reports of many other workers (Wogan, 1966; Hamilton et al., 1972; Osborne, 1981; Doerr et al., 1983; Manning et al., 1990a,b) in that dietary aflatoxin increased the liver lipid contents when diets were containing 30 IU vitamin E/kg. The reason why the liver lipid contents of rats fed aflatoxin-free diets were increased by higher levels of vitamin E is not clear at this moment.

TABLE 4. EFFECTS OF DIETARY AFLATOXIN, VITAMIN E, AND SE ON TOTAL LIPIDS AND CHOLESTEROL CONTENTS IN THE LIVER OF MALE RATS (N = 5)

Treatments			Total	Total	Free	Esterified
Aflatoxin	Vitamin E	Se	lipids	cholesterol	cholesterol	cholesterol[1]
mg/kg	IU/kg	mg/kg	%[2]	··· mg/100 g, liver wt ···		
0	30	0.1	15.0	237	144	87.3
		1.0	15.6	248	144	101.0
		2.0	15.6	243	142	82.0
	60	0.1	17.5	259	149	110.7
		1.0	18.1	267	156	110.3
		2.0	18.0	257	157	100.0
	120	0.1	19.2	266	158	108.3
		1.0	19.4	272	163	109.0
		2.0	19.0	273	165	108.0
1	30	0.1	20.0	205	117	88.0
		1.0	19.5	202	113	89.0
		2.0	18.6	209	113	96.3
	60	0.1	17.4	228	160	74.7
		1.0	16.8	231	159	72.7
		2.0	16.8	220	154	72.3
	120	0.1	17.1	228	159	69.7
		1.0	17.5	236	162	74.7
		2.0	16.9	227	163	64.0
LSD (.05)			2.62	22.3	16.5	24.6
Main effects						
Aflatoxin (AF)			*	*	*	*
Vitamin E (VE)			*	*	*	*
Se			NS	NS	NS	NS
VF × VE			*	NS	*	*
AF × Se			*	NS	NS	NS
VE × Se			NS	NS	NS	NS
AF × VE × Se			NS	NS	NS	NS

*$p < .05$.

[1]Values represent mg of esterified cholesterol (total cholesterol-free cholesterol) per 100 g of liver weight.

[2]Percent of liver dry weight.

The total cholesterol contents of the liver was significantly reduced by the feeding of aflatoxin, however, high levels of vitamin E in aflatoxin diets significantly increased the total cholesterol contents. The free cholesterol level in the liver was increased by feeding higher levels of vitamin E, and this tendency was more remarkable when rats were fed aflatoxin. Thus, the dietary aflatoxin seems to reduce the free cholesterol contents in the liver, and high levels of vitamin E alleviates this effect. However, the supplementation of Se did not exert any effect on either the lipid or cholesterol contents in the liver in the current experiment.

In conclusion, the extra dietary vitamin E or Se may partially alleviate some of the harmful effects of aflatoxin including decreased body weight, feed efficiency, GSH-Px activity, and hepatic cholesterol levels in rats.

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