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RESEARCH ARTICLE

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Protective effects of selenium and vitamin E on rats consuming maize naturally contaminated with mycotoxins

Abstract Protective effects of antioxidant additives of selenium and vitamin E on rats that consumed maize naturally contaminated with mycotoxins were explored in this paper. Thirty-two Wistar female rats were randomly divided into four groups. The control group was given the basic diet with normal maize. The contaminated maize group was given the diet in which normal maize was replaced by mycotoxin-contaminated maize. The selenium group and vitamin E group were respectively fed mycotoxin-contaminated diet supplemented with 0.4 mg·kg^{-1} selenium from yeast or 100 mg·kg^{-1} vitamin E. The trial lasted for 4 weeks. Compared with the control group, antioxidative status was decreased significantly in the contaminated maize group. However, the status in the selenium group and vitamin E group was increased significantly compared with the contaminated maize group. The activities of enzymes related to liver function were significantly higher in the contaminated maize group than those in the control group, whereas they were significantly lower in the selenium group and/or the vitamin E group compared to the contaminated maize group. It is concluded that selenium and vitamin E were able to alleviate oxidative stress and liver function damage due to the consumption of maize naturally contaminated with mycotoxins.

Keywords selenium, vitamin E, mycotoxin-contaminated maize, rats

1 Introduction

Mycotoxins are toxic and/or carcinogenic metabolites derived from mold development on grains. Mycotoxins are highly undesirable substances that should be prevented in food, and zero tolerance would be ideal for the purpose of protecting food from mycotoxins. However, grains, for instance maize, are often contaminated with mycotoxins either in the field or in storage (Galvano et al., 2005). Mycotoxins cause detrimental effects on both humans and farm animals, such as growth impairment, gastro-intestinal dysfunction, and immune depression (Dänicke et al., 2002; Swamy et al., 2004; Galvano et al., 2005; Su et al., 2006). The addition of mycotoxin adsorbents or binders is the usual means for protection against mycotoxin contamination of grains or other food, but the protective efficacy of adsorbents and/or binders is controversial at present.

On the other hand, a delicate balance between antioxidants and pro-oxidants in the body in general, and specifically in the cell, is responsible for the regulation of various metabolic pathways leading to the maintenance of immuno-competence, growth and development, as well as protection against stress conditions. Some nutritional stress factors have a negative impact on this antioxidant/pro-oxidant balance. In this respect mycotoxins are considered to be one of the most important feed-borne stress factors (Surai and Dvorska, 2005). It has been proven that mycotoxins can stimulate lipid peroxidation in the body, which is one of the underlying mechanisms of their toxic effect (Surai, 2006). Due to the antioxidative properties of selenium and vitamin E, they may counteract mycotoxicosis in humans and/or livestocks. However, there are limited scientific information and data on the protective effect of selenium and/or vitamin E against the changes induced by mycotoxins. Our study therefore aimed to examine the protective effects of antioxidant selenium and vitamin E on rats fed on the diet of maize naturally contaminated with mycotoxins.

2 Methods

2.1 Preparation of the contaminated maize

The maize was purchased from the same production area at the same time. Some of the maize was invaded by molds after half year storage at a moist depository. This kind of

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maize was regarded as the contaminated maize after crushing and mixture. The other normal maize stored at a dry depository was not invaded by fungi. Both the normal maize and the contaminated maize were subjected to determination of the proximate composition according to the methods of AOAC (1995).

2.2 Animals and management

Thirty-two experimental female albino rats of Wistar strain weighing 190–240 g per rat were bought from the Institute of Laboratory Animals, Sichuan Academy of Medical Science, China. All rats were housed individually in stainless-steel cages at a constant room temperature (20–25°C) with a 12-h light-dark cycle, and provided with water and feed *ad libitum*. The trial was carried out in the animal research base at the Institute of Animal Nutrition, Sichuan Agricultural University, China.

2.3 Experimental diets and design

The selenium enriched yeast used for this research was obtained from Alltech Biotechnology Co., Ltd., Beijing, China. The vitamin E was provided by Bayer Animal Health Co., Ltd., Chengdu, China. The diets were prepared based on the maize-soybean meal according to Reeves (1993). Four experimental diets were formulated as shown in Table 1. Dietary contents of total aflatoxin, deoxynivalenol, zearalenone, fumonisin B1, T-2 toxin, and ochratoxin A were analyzed using enzyme linked immunosorbent assay (ELISA) at the Feed Quality Supervision and Testing Centre of the Ministry of Agriculture of the People's Republic of China, Chengdu, China. The contents of the mycotoxins in the basic diet and contaminated diet are shown in Table 2. The rats were divided into four treatment groups according to body weight. There were 8 rats in each group. Each treatment group was further replicated 8 times with one rat per replicate. The groups were then allotted to four treatment diets in a Completely Randomized Design (CRD). Briefly, the control group (T1) was given the basic diet with normal maize; the mycotoxin-contaminated maize group (T2) was given the diet of contaminated maize instead of normal maize; the selenium enriched yeast group (T3) and the vitamin E group (T4) were fed the contaminated maize diet supplemented with 0.4 $mg \cdot kg^{-1}$ yeast selenium or 100 $mg \cdot kg^{-1}$ vitamin E, respectively. The trial lasted for 28 days.

2.4 Sample collection and analytical methods

The feed intake of rats was measured everyday. At the end of the experiment, all the rats were weighed and anesthetized. Blood samples were then collected from heart. The serum was prepared by centrifuging (at 4000 $r \cdot min^{-1}$ for 5 min) and immediate storing at -20°C.

Activities of superoxide dismutase (SOD), gluthathione peroxidase (GPX), glutamate-pyruvate transaminase (GPT), glutamic-oxalacetic transaminase (GOT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP), total antioxidation capacity (T-AOC), and concentration of malondialdehyde (MDA), nitric oxide (NO) in serum were determined by assay kit according to the manufacturer's instructions. The kits were purchased from Jiancheng Bioengineering Institute, Nanjing, China.

2.5 Statistical analysis

Data derived from our study were analyzed by one-way ANOVA using the GLM procedure of SAS 9.0 (SAS Inst., Inc., Cary, NC). Each rat was an experimental unit. Statements of statistical significance were based on $P < 0.05$.

3 Results

3.1 Growth performance of rats

The growth performances of rats in each group are shown in Table 3. There were no significant differences in body weight gain and average daily feed intake among groups. The rats consuming maize naturally contaminated with mycotoxins trended to decrease in body weight gain and feed intake compared with the control group, whereas supplementation of selenium enriched yeast and/or vitamin E improved the growth performance in rats consuming mycotoxin-contaminated maize.

3.2 Oxidative and antioxidative status in serum of rats

Table 4 shows the oxidative and antioxidative status in serum of rats. There was no significant difference in the concentration of MDA. Compared with the control group, activities of SOD, GPX, and T-AOC were decreased significantly in the mycotoxin-contaminated maize group ($P < 0.05$). The activities of SOD, GPX, and T-AOC in the group supplemented with selenium enriched yeast or vitamin E were increased significantly compared with mycotoxin-contaminated maize group ($P < 0.05$). The content of NO in the mycotoxin-contaminated maize group was significantly higher than in the other groups ($P < 0.05$), whereas that in the vitamin E group was also much higher than the control group ($P < 0.05$).

3.3 Biochemical measurements of liver functions in rats

The biochemical measurements of liver functions in serum of rats are shown in Table 5. The activities of GPT, GOT, LDH and ALP in the mycotoxin-contaminated maize group were significantly higher than those in the control group ($P < 0.05$). The activities of GPT, GOT, LDH and ALP in the group supplemented with selenium enriched

Table 1 Composition of experimental diets (as fed-basis)

items	diet/%			
	control (T1)	contaminated (T2)	selenium (T3)	vitamin E (T4)
ingredient/%				
maize	65.20	—	—	—
contaminated maize[a)]	—	65.20	65.20	65.20
soybean meal	27.86	27.86	27.84	27.82
soybean oil	2.20	2.20	2.20	2.20
calcium-phosphorus	1.70	1.70	1.70	1.70
limestone	1.40	1.40	1.40	1.40
L-lysine	0.55	0.55	0.55	0.55
DL-methionine	0.23	0.23	0.23	0.23
iodized salt	0.20	0.20	0.20	0.20
choline chloride	0.10	0.10	0.10	0.10
flavour additive	0.02	0.02	0.02	0.02
vitamin mixture[b)]	0.04	0.04	0.04	0.04
mineral mixture[c)]	0.50	0.50	0.50	0.50
vitamin E	—	—	0.02	—
selenium enriched yeast	—	—	—	0.04
nutrient composition				
digestive energy/MJ·kg^{-1}	14.12	14.12	14.12	14.11
crude protein /%	18.00	18.13	18.13	18.11
crude fibre / %	2.49	2.49	2.49	2.49
calcium /%	1.00	1.00	1.00	1.00
phosphorus /%	0.65	0.65	0.65	0.65
lysine /%	1.32	1.32	1.32	1.32
methionine + cystine /%	0.79	0.79	0.79	0.79

Note: a) The proximate composition of normal and mycotoxin-contaminated maize was analyzed according to 1AOAC (1995). The crude protein in normal maize was 7.8%, whereas that in mycotoxin-contaminated maize was 8.0%. Other nutrients were equal between the two kinds of maize. (b) A diet per kg contains 14000 IU vitamin A, 1500 IU vitamin D_3, 120 IU vitamin E, 5 mg vitamin K, 13 mg vitamin B_1, 12 mg riboflavin, 12 mg vitamin B_6, 60 mg nicotinic acid, 24 mg panthotenic acid, 6 mg folic acid, 0.2 mg biotin, 0.05 mg vitamin B_{12}, 1250 mg choline, 460 mg vitamin C. (c) Nutrition composition per kg of diet includes 120 mg iron, 30 mg zinc, 10 mg copper, 75 mg manganese, 0.15 mg selenium, 0.5 mg iodine, 507 mg magnesium, 1.2 g potassium.

Table 2 Mycotoxin content of experimental diets (as fed-basis)

items	basic diet/mg·kg^{-1}	mycotoxin-contaminated diet/mg·kg^{-1}
aflatoxin	ND	ND
deoxynivalenol	1.10	1.02
nearalenone	ND	ND
fumonisin	3.50	3.44
T–2 toxin	0.28	0.45
ochratoxin	1.00	6.10

Note: ND means “cannot be detected”.

Table 3 Growth measurement of rats

growth measurements	T1/g	T2/g	T3/g	T4/g	SEM
body weight gain	20.85	20.54	21.70	21.45	0.54
average daily feed intake	21.10	20.78	21.98	21.64	0.55

Table 4 The antioxidant status in serum of rats

antioxidant status	T1	T2	T3	T4	SEM
MDA/nmol·mL^{-1}	3.17	3.46	2.85	3.50	0.26
SOD/U·mL^{-1}	263.51	182.53a	267.59b	218.14c	2.10
GPX/U	1573.68	542.11a	1205.26c	1271.05c	46.79
T-AOC/U·mL^{-1}	5.78	4.50	6.27	8.67c	0.66
NO/μmol·mL^{-1}	8.96	50.00a	7.51b	39.31c	1.04

Note: Letter "a" denotes $P < 0.05$ compared with T1; letter "b" denotes $P < 0.05$ compared with T2; and letter "c" denotes $P < 0.05$ compared with both T1 and T2.

Table 5 Biochemical measurements of liver functions in serum of rats

biochemical measurements	T1	T2	T3	T4	SEM
GPT/U·mL^{-1}	5.75	22.61a	6.23b	7.46b	1.95
GOT/U·mL^{-1}	30.45	53.05a	24.35b	39.24c	2.83
LDH/U·L^{-1}	3110.3	4821.19a	2403.97c	3823.40c	107.21
ALP/U·100mL^{-1}	6.08	7.39a	2.15c	5.98b	0.35

Note: Letter "a" denotes $P < 0.05$ compared with T1; letter "b" denotes $P < 0.05$ compared with T2; and letter "c" denotes $P < 0.05$ compared with both T1 and T2.

yeast and/or vitamin E were significantly lower than those in the mycotoxin-contaminated corn group ($P < 0.05$).

4 Discussion

There were no significant differences in the body weight gain and the average daily feed intake among the groups, which is different from some previous researches (Swamy et al., 2003; Swamy et al., 2004; Su et al., 2006). The reasons may include three aspects: First, animals consuming the mycotoxin-contaminated diets adapted metabolically to restricted peripheral blood circulation in order to reduce heat loss and improve nutrient utilization (Rotter et al., 1994). Second, due to the improvement in crude protein in mycotoxin-contaminated maize (from 7.8% to 8.0%), it was speculated that some substances that could not be used, even anti-nutritional factors, were transformed to microbial proteins by molds. Thus, the feed utilization in rats was increased. Third, there was a small portion of mycotoxin in the basic diet (Table 2), and although the amount was much lower than the international standard, it might also have reduced the growth performance of the control group.

In our study, it was clear that the content of T–2 toxin and ochratoxin A (OTA) in the mycotoxin-contaminated diet was much higher than those in the basic diet (Table 2). Both T–2 toxin and OTA were able to induce oxidative stress and injury via lipid peroxidation (Surai and Dvorska, 2005). The consumption of maize naturally contaminated with mycotoxins caused depression of activities of SOD, GPX and T-AOC, whereas concentration of MDA was not significant among the groups. These indicated that mycotoxins induced oxidative stress due to the damage in the anti-oxidative system in the animals' body. Our results were similar to the report of Meki and Hussein (2001). However, some researchers showed that OTA and T-2 toxin significantly increased the content of thiobarbituric acid-reactive substances (TBARS) and MDA (Gautier et al., 2001; Vila et al, 2002). Therefore, mycotoxins may affect both sides of the antioxidant/pro-oxidant balance. They could not only damage the anti-oxidative system in body, but may also stimulate lipid peroxidation. The activities of SOD, GPX and T-AOC were recovered when selenium enriched yeast and/or vitamin E was supplemented in the mycotoxin-contaminated diet. These findings revealed that both selenium and vitamin E could alleviate the oxidative stress caused by mycotoxin-contaminated maize.

It was well known that OTA was a strong hepatotoxic mycotoxin (Hussein and Brasel, 2001). This was similar to the findings in our study. We also found that OTA in mycotoxin-contaminated maize caused damage to the liver functions in rats. However, the addition of selenium enriched yeast and/or vitamin E was able to protect the liver function in animals consuming mycotoxin-contaminated maize.

The concentration of nitric oxide (NO) in themycotoxin-contaminated maize group that was five times higher than that in the control group was also observed. It was significantly reduced when adding selenium enriched yeast and/or vitamin E. NO is a relative steady gas-free radical. While it reacts with inherent O_2^- in animals' body, a strong oxidative substance $ONOO^-$ would be produced.

Subsequently, $ONOO^-$ could be decomposed into some toxic products, such as OH^-, $OH\cdot$, NO_2^- and NO_3^-. These free radicals could result in oxidative stress in animals (Zhong and Sun, 1997). On the other hand, NO could interfere with the enzymes related with biological transformation in the liver. The improvement of NO production could cause turbulence in the metabolism in the liver (Curran et al., 1991; Harbrechet et al., 1992). Thereby, the underlying mechanism for mycotoxins damaging liver function in animals might be related with the volcanic improvement of NO levels, and inducible nitric oxide synthase (iNOS) might be a key enzyme in this pathway.

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