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THE EFFECTS OF AFLATOXIN AND GLUCOMANNAN ON SOME ANTIOXIDANTS AND BIOCHEMICAL PARAMETERS IN RABBITS

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In this study the effects of aflatoxin added to the feed and the effects of glucomannan supplemented in order to prevent aflatoxin absorbtion on some biochemical parameters and antioxidants were studied in 40 New Zeland rabbits. The rabbits were randomly alloted to the following groups: control (C), glucomannan (G), glucomannan + aflatoxin (AG) and aflatoxin (A). At the end of the ten weeks trial MDA levels increased, GSH and SOD levels were decreased ($p<0.05$) in group A compared with control group levels. Cholesterol, glucose, albumin and total protein levels also decreased, AST and ALT levels were increased in group A compared with the control group. In the group G the former parameters were not affected by glucomannan application alone. On the other hand in the AG group MDA, GSH, SOD, cholesterol, glucose, albumin and total protein levels were higher, while AST and ALT levels were lower compared with the control levels, although these differences were not significant. In conclusion, the results determined in the study might be important to demonstrate the effects of aflatoxicosis on some biochemical and antioxidant parameters.

Key words: aflatoxin, glucomannan, rabbit, biochemical parameters, antioxidant

INTRODUCTION

One of the most important effects of unconditioned storage of food and foodstuff is intoxication caused by mycotoxins (Çelik *et al*., 2000a). Among mycotoxins, aflatoxins are the most common and aflatoxin B1 is the most harmful one (Abdel-Wahab *et al*., 2002; Eraslan *et al*., 2004). Consumption of aflatoxin contaminated food by man and animals can cause important health problems, as well as important economical losses. Aflatoxicosis can cause several defects in organs and tissues, a decrease in growth rate, an increase in death rate, immunosuppression, anemia, increased coagulation time and deteriorated lipid, carbohydrate and protein metabolism (Çelik *et al*., 1996; Raju and Devegowda, 2000).

Aflatoxins are most dangerous mycotoxins both for humans and animals (McKean *et al*., 2006). Aflatoxin B1 is mitogenic, hepatotoxic, hepatocarcinogenic and causes oxidative stress (Çelik *et al*., 2000b; Meki *et al*., 2004). In the living body, environmental factors show their toxic effects mostly through the formation of oxygen radicals. When free radicals are formed overcame the protective effects of the defense system, they can affect the metabolic pathways (Kilinç, 1986; Erenel *et al*. 1992).

As the result of toxic effects of aflatoxin biochemical and hematological parameters have been reported to be altered. In chronic and sub-clinical aflatoxicosis cases, changes in biochemical and hematological parameters occur before clinical symptoms develop (Aravind *et al*., 2003). AF is thought to inhibit protein synthesis and reduce blood protein levels. As a result of intoxications with aflatoxins total protein, cholesterol, triglyceride, and glucose levels have been reported to decrease significantly (Kubena *et al*., 1993). As a matter of fact, Rosa *et al.* (2001) studied the effects of aflatoxin in broilers and found a reduction in total protein, albumin, total cholesterol, uric acid, inorganic phosphorus and calcium levels. Furthermore Aravind *et al.* (2003) gave aflatoxin at different levels to broilers and found a reduction in total protein, albumin, total cholesterol, glucose and triglyceride levels.

In the investigation of preventive application against negative effects of aflatoxin, the most popular method is to add compounds that have a binding character aflatoxin and easy application. The main mechanism of function of these compounds is to bind aflatoxin present in the food irreversibly and therefore, limit its absorption from the digestive system (Eraslan *et al*., 2004). In order to reutilize foods contaminated with aflatoxin, some adsorbants can be supplemented to the contaminated feed (Harvey *et al*., 1991; Kubena *et al*., 1993). A successful detoxification procedure should be economic, and should eliminate all the residues of toxin without leaving any harmful remnants without impairing food quality. In recent years, studies on natural and synthetic zeolits, aliminium silicas, bentonites, fillosilicas and klinoptilotites have been performed. A different and new approach in biological detoxification is the usage of Saccharomyces cerevisiae (SCE) and its cell wall membrane component (glucomannan) to reduce side effects of aflatoxin (Raju and Devegowda, 2000; Parlat *et al*., 2001; Aravind *et al*., 2003).

In the present study, the effects of aflatoxin on some biochemical parameters and the antioxidant system were determined in rabbits fed feed containing aflatoxin. Furthermore, the effect of adding glucomannan, which is a known aflatoxin binder, on the negative effects of aflatoxin were studied, as well.

MATERIALS AND METHODS

In the present study, 40 healthy New Zaland rabbits were included. The rabbits were divided into 4 equal groups. The average body weights were comparable between groups. Each rabbits was kept in individual cages and during the experiment the rabbits received clean water and were fed *ad libitum* as described below:

Group 1 (C); fed with pelleted food for rabbits.

Group 2 (A); fed with pelleted food containing 125 ppb aflatoxin

Group 3 (G); fed with pelleted food containing 1000 ppm glucomannan.

Group 4 (AG); fed with pelleted food containing 1000 ppm glucomannan + 125 ppb aflatoxin.

The rabbits received appropriate care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health.

At the end of the experiment, by means of cardiac puncture, blood samples were collected into tubes with added anticoagulant (3.8% sodium citrate) and without anticoagulant. Serum samples, plasma samples and erythrocyte packets were obtained from blood samples. MDA present in packed erythrocytes reacts with TBA. The so formed compound has an absorbance peak at 532 and 600 nm. MDA concentration values can be calculated through the obtained absorbance values (Slater, 1984; Akkus I., 1995). Whole blood GSH level was determined by the method described by Beutler *et al*. (1963). Plasma total protein, cholesterol, albumin, SOD, AST and ALT levels were determined by commercial kits (Biosystem) spectrophotometrically (Chebios Optimum-one UV-VIS).

Statistical differences among the groups were tested by analysis of variance (ANOVA) followed by Duncan's test using SPSS for Windows version 10.0. Significance was considered at $p<0.05$.

RESULTS AND DISCUSSION

Aflatoxins (AF) are mitogenic, hepatotoxic and hepatocarcinogenic both for humans and animals and cause oxidative stress (Meki *et al*., 2004). Even small amounts of AF are dangerous for animal health because of detrimental effects on some biochemical parametrs (Keçeci *et al*., 1998).

Oxidative damage mainly causes dysfunction of cellular components such as enzymes, nucleic acids, membranes and proteins (Rastogi *et al*., 2001). In the present study, to describe cellular lipid damage caused by aflatoxin, MDA values were determined, although MDA level was higher in group A compared to control group, it was not significant (Table 1). GSH level decreased in group AG and group A, compared to the control group (Table 1). Furthermore SOD values were decreased in group A compared, but significantly only when compared to the control group. Similarly, Choudhary and Verma (2005) studied aflatoxicosis in mice and found increased lipid peroxidation and decreased nonenzymatic antioxidants such as glutathione, ascorbic acid and enzymatic antioxidants such as superoxide dismutase glutathione peroxidase and catalase. Furthermore, Rastogi *et al.* (2001) also reported that aflatoxin causes reduction in SOD, glutathione- S-transferase, glutathione peroxidase and glutathione reductase activities. These findings supported the idea of reactive oxygen as a mean of citotoxic effect of aflatoxin.

Serum biochemical analysis (Table 2) revealed that treatment with AF and AG significantly increased blood levels of ALT, AST and cholesterol, while decreased blood levels of total protein, albumin and glucose. In intoxicated

rabbits, decreased total protein, albumin and glucose concentrations were in agreement with Keçeci *et al*. (1998) and Oguz *et al*. (2000) findings. In addition Yousef *et al*. (2003) described decreased total proteins, albumin and glucose concentrations and increased ALT and AST activities in rabbits with aflatoxicosis. Soliman *et al*. (2001) found an increase in AST, but no changes in serum total protein, albumin concentration in rabbits with aflatoxicosis. Furthermore Abdel Wahab *et al*. (2002) studied aflatoxicosis in rats and described a decrease in total protein, albumin, cholesterol and triglyceride levels, but increased ALT and AST were determined. Our findings support the above studies. The decreased serum total protein, albumin, cholesterol, triglyceride and glucose values and increased ALT and AST activity observed in the studies were due to the hepatotoxic effect of AF characterized by the inhibition of protein synthesis and impairment of carbohydrate and lipid metabolism (Arawind *et al*., 2003; Basmacioglu *et al*., 2005).

Table 1. Mean MDA, GSH and plasma SOD values obtained from control and experimental groups (n =10, X ± SX)

Parameters	C	G	AG	A
MDA (nmol/l)	3.93 ± 0.45	4.36 ± 050	4.46 ± 0.63	4.87 ± 0.54
GSH (mg/dl)	33.61 ± 1.92^{a}	32.63 ± 0.46^{a}	25.17 ± 0.72^{b}	21.92 ± 0.72^{b}
SOD (U/ml)	0.278 ± 0.04^{a}	0.272 ± 0.06^{ab}	0.263 ± 0.06^{ab}	0.255 ± 0.04^{b}

a, b; $p<0.05$.

Table 2. Biochemical parameters in control and experimental groups (n =10, X ± SX)

Parameters	C	G	AG	A
Cholesterol (mg/dl)	85.17 ± 3.00^{a}	86.50 ± 1.31^{a}	72.19 ± 2.40^{b}	71.70 ± 1.44^{b}
Glucose (mg/dl)	158.28 ± 7.11^{a}	151.78 ± 3.41^{ab}	142.66 ± 3.72^{b}	138.35 ± 3.84^{b}
Albumin (g/l)	3.52 ± 0.44^{a}	3.48 ± 0.56^{ab}	3.36 ± 0.51^{bc}	3.25 ± 0.43^{c}
T.Protein (g/l)	6.08 ± 0.46^{a}	5.86 ± 1.01^{ab}	5.74 ± 0.64^{b}	5.79 ± 0.96^{b}
AST (U/L)	28.03 ± 1.66^{b}	29.82 ± 1.39^{b}	36.43 ± 3.09^{a}	38.43 ± 2.44^{a}
ALT (U/L)	14.95 ± 0.26^{b}	17.81 ± 1.40^{ab}	19.03 ± 1.37^{ab}	21.39 ± 2.35^{a}

a, b, c; $p<0.05$.

Extensive research has been conducted to counter mycotoxicosis by physical, chemical, nutritional or biological approaches (Raju and Devegowda, 2000). In the present study, for this purpose glucomannan was added to the animals' feed. Eraslan *et al*. (2004) and Banlura *et al*. (2005) reported that esterified glucomannanes got rid of toxic effects of AF in broilers. Again in other study in broilers, esterified glucomannan decreased negative affects of AF on some biochemical parameters and body weight gain (Arawind *et al*., 2003). In the present study, in group G the biochemical parameters and the antioxidant system were not significantly changed compared with control group (Table 1, 2). In AG group, although not statistically significant, MDA, GSH, SOD, cholesterol, glucose, albumin and total protein were higher, AST and ALT values were lower than the same values obtained from group A. Furthermore, apart from GSH and AST levels in other parameters were not different from group G. For this reason, values obtained from AG can not be argued exactly in the same fashion as those obtained in group G; however the used dose is thought to be effective. None the less different doses of glucomannan should be tested in future studies.

The effects of aflatoxin on both antioxidant system and biochemical parameters depend on the amount of aflatoxin, animal species and duration of toxicity. As a result, findings presented in this study contribute to the overall literature and also chronic intoxications can be diagnosed before clinical sings occurre. The effectiveness of glucomannan at this dose is believed to be important.

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UTICAJ AFLATOKSINA I GLUKOMANANA NA NEKE ANTIOKSIDATIVNE I BIOHEMIJSKE PARAMETRE KUNIĆA

DÖNMEZ N i KESKIN E

SADRŽAJ

U ovoj studiji je proučavan uticaj glukomanana dodatih u obrok kunića sa i bez prisustva aflatoksina. Ogled je izveden na 40 Novozelandskih belih kunića podeljenih u četiri jednake grupe: kontrolna (C), glukomanan (G), glukomanan + aflatoksin (AG) i aflatoksin (A). Po isteku perioda od 10 nedelja nivo MDA je bio povećan, a aktivnost GSH i SOD smanjena u grupi A u odnosu na vrednosti registrovane u kontrolnoj grupi C ($p<0,05$). Koncentracija holesterola, glukoze, albumina i ukupnih proteina je bila smanjena, a aktivnost enzima AST i ALT povećana u grupi A u poređenju sa kontrolnom grupom. U grupi G ispitivani parametri nisu bili izmenjeni dodavanjem glukomanana u obrok. Na suprot ovome, u grupi AG, koncentracija MDA, aktivnost GSH i SOD, koncentracija holesterola, glukoze, albumina i ukupnih proteina su bile veće a aktivnost AST i ALT manja u poređenju sa kontrolama, mada ove razlike nisu bile statistički značajne. Rezultati ove studije ukazuju na promene vrednosti nekih biohemijskih i antioksidativnih parametara tokom aflatoksikoze.