

The Effects of Yeast Glucomannan (Mycosorb) on Lipid Peroxidation and Non-Enzymatic Antioxidative Status in Experimentally Induced Aflatoxicosis in Broilers

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Abstract: The aim of this study was to determine the effect of yeast glucomannan (Mycosorb), an aflatoxin binder, on lipid peroxidation and non-enzymatic antioxidative status in experimentally induced aflatoxicosis in broilers. Forty, 1-day-old male broiler (Ross 308) chickens were randomly divided into 4 groups of 10 birds each. The control group was fed a basal diet and the remaining groups received 0.75 g kg⁻¹ Mycosorb, 2 ppm Aflatoxin (AF) and 0.75 g kg⁻¹ Mycosorb +2 ppm Aflatoxin (AF) for 21 days. At the end of day 21, blood samples were collected and plasma Malondialdehyde (MDA), Ceruloplasmin (CP), albumin (alb), uric acid, vitamin A (vit A), β -carotene, vitamin C (vit C) and vitamin E (vit E) levels were determined. Plasma MDA levels increased insignificantly and alb levels decreased significantly in AF and AF+Mycosorb groups when compared to control and mycosorb groups. Plasma CP, vit A and β -carotene levels decreased significantly in AF groups when compared to control. In AF and AF+Mycosorb group, significant decreases were determined in vit E levels when compared to control and in vit C levels when compared to mycosorb group. Plasma uric acid levels were not affected by these treatments. It is concluded that subacute dietary intake of AF altered nonenzymatic components (CP, alb, vit A, β carotene, Vit E) of antioxidant defense systems and 0,75 g kg⁻¹ yeast glucomannan was not sufficient to ameliorate the oxidative damage caused by AF in broilers.

Key words: Aflatoxicosis, broiler, lipid peroxidation, mycosorb, non-enzymatic antioxidant

INTRODUCTION

Two hundred and fifty, 300 different species of fungi are known to produce mycotoxin till now and 20-25 mycotoxin group are found as a natural pollutant on feed and feedstuffs Aflatoxins, produced by *Aspergillus flavus* and *Aspergillus parasiticus*, are the most frequently encountered mycotoxins in feed and feedstuffs. They lead considerable economic losses in poultry sector due to naturally contaminated feedstuffs (Kaya, 2002).

There are many toxic effects of AF, one of these is lipid peroxidation (Rastogi *et al.*, 2001; Eraslan *et al.*, 2004, 2005a,b; Preetha *et al.*, 2006; Umarani *et al.*, 2008). The main manifestation of oxidative damage is lipid peroxidation. It plays an important role in toxicity (Rastogi *et al.*, 2001; Umarani *et al.*, 2008). From the studies conducted on poultry it has been found that the

most affected organ is liver following the administration of the toxin (Ortatatli and Oguz, 2001; Karaman *et al.*, 2005). Therefore, aflatoxins are hepatotoxic (Preetha *et al.*, 2006; Ortatatli and orguz, 2001; Karaman *et al.*, 2005) and hepatocarcinogenic agents. They cause free radical producing during hepatic cells damage (Preetha *et al.*, 2006). These radicals initiate lipid peroxidation, since all cell membranes contain the fatty acids which are substrates for such a reaction (Rastogi *et al.*, 2001). This is thought to be the mechanism underlying the hepatotoxicity of aflatoxins (Preetha *et al.*, 2006). Aflatoxins are not directly toxic by themselves, they become effective when transform into AFB1-8,9 epoxide by cytochrome P-450 in the liver (Coulombe, 1993). Probably an increase in AFB1-8, 9-epoxide causes increases in hepatic lipid peroxide level (Toskulkao and Glinsukon, 1988).

Antioxidants are substances that protect cells against the effects of free radicals. Antioxidants inhibit lipid peroxidation by breaking the chain reactions and scavenging initiating free radicals (Dundar and Aslan, 1999). Antioxidants are classified as enzymatic and non-enzymatic antioxidants. Non enzymatic antioxidants are α -tocopherol (Vitamin E), β -carotene, vitamin A, ubiquinols, ascorbate, glutathione, melatonin, cysteine, ceruloplasmin, haemoglobin, bilirubin, albumin and other compounds. Enzymatic antioxidants are superoxide dismutase, catalase, glutathione S transferase, glutathione peroxidase (Akkus, 1995).

It is important to prevent the feed and feedstuffs from AF contamination. One of the promising and practical way is to use adsorbents (Huwig *et al.*, 2001). Adsorbents bind to AF in the gastrointestinal system and decrease the rate of AF absorbed (Diaz *et al.*, 2002).

Yeast glucomannan is a natural toxin binder. Yeast glucomannan has been found to counteract the toxic effect of aflatoxin in feed of growing broiler chicken (Ortatatli and Oguz, 2001; Karaman *et al.*, 2005; Raju and Devegowda, 2000; Basmacioglu *et al.*, 2005; Safameher and Shivazad, 2007). Karaman *et al.* (2005) showed that Yeast glucomannan (Mycosorb) alleviated the adverse effects of aflatoxin on the pathological changes and that the higher concentration of yeast glucomannan (1 g kg⁻¹) was more effective than the lower concentration (0.5 g kg⁻¹) and mycosorb itself had no adverse effect.

On different species, there have been many reports indicating the detrimental effects of AF (Eraslan *et al.*, 2004, 2005b, Ortatatli and Orguz, 2001; Cam *et al.*, 2008) and the protective effect of adsorbent from AF toxication (Eraslan *et al.*, 2004, Ortatatli and Orguz, 2001; Karaman *et al.*, 2005; Raju and Devegowda, 2000; Basmacioglu *et al.*, 2005; Safameher and Shivazad, 2007; Sehu *et al.*, 2005; Jansen *et al.*, 2006). However, there is limited number of studies about oxidative stress of aflatoxicated broilers (Eraslan *et al.*, 2004, 2005).

Therefore, the objective of this study was determine the efficacy of aflatoxin in feed on lipid peroxidation and some non-enzymatic components of antioxidant defense system. In addition, to evaluate the role of the efficacy of Mycosorb at 0.75 g kg⁻¹ to prevent the adverse effects of AF in contaminated feed in broiler chicken.

MATERIALS AND METHODS

Aflatoxin was produced, by the method described by Shotwell *et al.* (1966) modified by Demet *et al.* (1995), from the strain of *Aspergillus parasiticus* NRLL 2999 (National Center for Agricultural Utilization Research, Peoria, IL, USA) on rice. Immunoaffinity columns (Vicom AflaTest-Affinity Column) were used to analyse the

aflatoxin content of the culture material and quantified via high performance liquid chromatography (HPLC) (Agilent 1100 Series) The AFs B₁, B₂, G₁ and G₂ standards were purchased from Sigma-Aldrich (Taufkirchen, Germany). The stock solutions, working standards and the calibration curve were prepared as described by Stroka *et al.* (2000). The total amount of AFs (AF B₁, B₂, G₁ and G₂) were 90.35 mg kg⁻¹ in rice powder and consisted of 83.33% AF B₁, 10.42% AF G₁, 4.17% AF B₂ and 2.08% AF G₂, based on the total AF in the rice powder.

Experimental design and management: In this study, 41 days old Ross 308 broiler chicks were distributed by similar body weight to four groups. The control group was fed a basal diet and the remaining groups received 0.75 g kg⁻¹ Mycosorb, 2 ppm AF and 2 ppm AF+0.75 g kg⁻¹ Mycosorb for 21 days. The chicks were housed in electrically heated batteries under fluorescent lighting and feed and water was supplied *ad libitum*. This study was approved by Ethics Committee of University of Kırıkkale, Faculty of Veterinary Medicine (Approval number, 17. 03. 2006-06/03).

Biochemical assays: At the end of the 21 day treatment period, blood samples were collected into heparinized test tubes from wein vena to determine lipid peroxidation and some non-antioxidant enzyme components. Plasma samples were separated by centrifugation at 3000 rpm for 10 min and stored -30°C until the analysis. Lipid peroxidation levels were measured with Thiobarbituric Acid Reactions (TBARs) in plasma by the method of Moreno *et al.* (2003). The values of TBARs material were expressed in terms of MDA. Plasma albumin (TECO, USA) and uric acid (DDS, Germany) levels were determined by a Shimadzu UV 1700 spectrophotometer using commercial kits. Plasma CP, vit A, vit C and vit E levels were determined by the methods of Colombo and Richeric (1964), Suzuki and Katoh (1990), Haag (1985) and Martinek (1964), respectively.

Statistical analysis: Statistical analysis of data were performed using the by SPSS 13. 0 version for Windows. One-way Analysis of Variance (ANOVA) was used for the differences between groups. When the F values were significant, Duncan's Multiple Range Test was performed. All data were expressed as means \pm SEM.

RESULTS AND DISCUSSION

Aflatoxins were reported to produce free radicals. The free radicals generated by AFB₁, can be important mediators of damage to cell structures, nucleic acids,

Table 1: Plasma MDA and some non-enzymatic antioxidant levels of control and treatment groups in experimentally induced aflatoxicosis in broilers (n=10)

Parameters	Group				p
	Control	Mycosorb	AF	AF+Mycosorb	
MDA ($\mu\text{mol L}^{-1}$)	1.95±0.36	1.80±0.45	2.44±0.63	2.23±0.52	-
CP (mg dL ⁻¹)	2.76±0.46 ^a	2.13±0.17 ^{ab}	1.56±0.29 ^b	2.35±0.13 ^{ab}	*
Uric acid (mg dL ⁻¹)	6.23±0.45	5.66±0.61	4.98±0.68	5.38±0.61	-
Alb (g dL ⁻¹)	0.83±0.03 ^a	0.96±0.03 ^a	0.43±0.05 ^b	0.46±0.06 ^b	***
Vit A ($\mu\text{g dL}^{-1}$)	137.02±16.23 ^a	111.92±17.78 ^{ab}	82.60±12.98 ^b	87.14±7.64 ^b	*
β -carotene ($\mu\text{g dL}^{-1}$)	187.54±11.31 ^a	166.20±9.35 ^{ab}	121.97±9.12 ^c	137.67±12.50 ^{bc}	***
Vit C ($\mu\text{g mL}^{-1}$)	15.87±0.51 ^{ab}	18.61±0.86 ^a	14.75±1.32 ^b	14.81±0.97 ^b	*
Vit E (mg dL ⁻¹)	3.64±0.20 ^a	3.52±0.15 ^{ab}	2.81±0.21 ^b	2.91±0.34 ^b	*

^{a-c}: Values within each row with different superscripts differ significantly, -:not significant ($p>0.05$), *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$

lipids and proteins and then expected to induce lipid peroxidation (Rastogi *et al.*, 2001; Umarani *et al.*, 2008). Antioxidants prevent Reactive Oxygen Species (ROS) concentrations from reaching a high-enough level within a cell that damage may occur (Seifried *et al.*, 2007).

In this study, 2 ppm AF increased the level of plasma MDA slightly as an indicator of lipid peroxidation and this increase was statistically insignificant ($p>0.05$) in comparison to the control and 0.75 g kg⁻¹ mycosorb given groups (Table 1). Eraslan *et al.* (2005a) showed that erythrocyte MDA levels in broilers increased slightly in the groups administered 0.5 and 1 ppm AF on days 15 and 30. But these increases were statistically insignificant, whereas, these increases were found to be statistically significant on day 45. Eraslan *et al.* (2005a) suggested that the increase of MDA level has been observed at high doses and known to depend on the exposure period.

In this study, the levels of CP decreased significantly ($p<0.05$) in AF treatment group when compared to control and alb levels also decreased significantly ($p<0.001$) in AF and AF+ Mycosorb groups when compared to control and mycosorb groups. However, uric acid levels were not affected by these treatments (Table 1). The significant decreases in the level of alb of aflatoxicated group than to control are in agreement with the studies on broiler chickens (Basmacioğlu *et al.*, 2005; Safameher and Shivazad, 2007). Ceruloplasmin and alb are proteins synthesized in the liver (Johnson *et al.*, 1999). Aflatoxin can cause damage to the liver (Preetha *et al.*, 2006; Ortatatli and Oguz, 2001; Coulombe, 1993). Thus levels of these protein synthesized in the liver may be decreased. The decrease of alb level may be due to the hepatic protein inhibition, a characteristic indicator of hepatotoxic effect of AF (Fernandez *et al.*, 1994). Ceruloplasmin has antioxidative properties in biological systems. It can protect polyunsaturated fatty acids in cellular membranes from ROS (Kim *et al.*, 1998). Ceruloplasmin and alb have the ability to bind copper ion in organisms. Binded copper ions could not catalyze free radical reactions. The antioxidants such as CP and alb show their activity by inhibiting the encountering of free radicals, which find in

interstitial fluids, to a catalyzer metal ion like copper (Aslan and Dundar, 2000). Also decreasing of CP and alb in the plasma may cause an increase in free copper in organism and therefore, lead to deficiency of the defense system against lipid peroxidation (Kohen and Nyska, 2002; Agli *et al.*, 1995). Copper ion does not initiate the lipid peroxidation, in fact it catalyzes the chain reaction of lipid peroxidation and degradation of the synthesized lipid hydroperoxides, so the free radicals become more harmful (Akkus, 1995).

The levels of Vit A decreased ($p<0.05$) significantly in AF treatment group when compared to control in this study (Table 1). There have been many reports indicated that AF administration effected the levels of serum Vit A and β -carotene in broilers (Salmanoğlu, 2002; Altıntaş *et al.*, 2003). Salmanoğlu (2002) reported that AF decreased the levels of serum Vitamin A and β -carotene on 21 days of broilers. Our results agree with the findings of Salmanoğlu (2002). In some studies (Ergun *et al.*, 2006; Sehu *et al.*, 2007) it was indicated that there is a decrease in the number of ribosomes in hepatocytes and this lead to a decrease in protein synthesis. Thus, a decrease may be seen in the retinol binding protein, a protein that carries Vit A inside and outside of the cell (Salmanoğlu, 2002). AFB₁ can reduce the level of Vit A *in vivo* and this effect on Vit A, is correlative with induction of hepatic microsome cytochrome P 450 (Liu and Zhou, 1989).

Significantly decreases ($p<0.05$) were obtained in the vit C levels in AF and AF+ Mycosorb group than to mycosorb group and the level of β -carotene also decreased significantly ($p<0.001$) in AF treatment group when compared to both control and Mycosorb treatment group in this study (Table 1). Vitamin C is the most important antioxidant found in extracellular fluids. It can protect biomembranes against lipid peroxidation damage by eliminating peroxy radicals before the latter can initiate peroxidation (McDowell, 2002). Vitamin C shows antioxidant activity by enhancing the immunity via maintaining the functional and structural integrity of important immune cells (McDowell, 2002). Aflatoxins inhibit immune function (Oguz *et al.*, 2003). This may be

the reason why Vit C and β -carotene levels were decreased in all aflatoxin given groups. Yeast glucomannan was found to have beneficial effects on immune response in broilers exposed to AF (Stanley *et al.*, 1993). Beta carotenes are known to stimulate immune function (Kalaycioglu *et al.*, 2000). This can explain the reason why the levels of Vit C and β - carotene was high in Mycosorb given group.

Vitamin E is one of the major antioxidants. It can limit lipid peroxidation by terminating chain reactions initiate in the membrane lipids (McDowell, 2000). Significant decreases ($p<0.05$) were determined in vit E in AF and AF+Mycosorb group than to control in this study (Table 1). It might be due to the excessive utilization of vitamin E for scavenging free radicals produced under these conditions (Umarani *et al.*, 2008). We could not find any study about the level of plasma Vitamin E and C in aflatoxicated animals. However, decrease of some antioxidant vitamin levels of aflatoxin treatment group may be related to increased utilization for scavenging of ROS (2008). Wilson *et al.* (1975) reported that damage caused by aflatoxin to hepatic cells, had decreased the synthesis of bile salts, which would affect the absorption of the fat-soluble vitamins. Also AF is known to cause damage in the gastrointestinal system. This may impair the intestinal adsorption and increase excretion. Thus, AF may decrease vitamin absorption and reduce their levels in body and hence weaken the antioxidant defense mechanism (Eraslan *et al.*, 2005).

CONCLUSION

The results of this study showed that subacute toxication of 2 ppm AF in feed did not cause lipid peroxidation significantly, however decreased some non-enzymatic antioxidants and 0.75 g kg^{-1} Mycosorb was not sufficient to prevent the adverse effects of AF in contaminated feed in broilers.

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