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The effects of aflatoxin and glucomannan on coagulation parameters in rabbits

Nurcan Dönmez*, and Ercan Keskin

University of Selcuk, Faculty of Veterinary Medicine, Department of Physiology, Konya, Turkey

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ABSTRACT

The aim of this study was to evaluate the effects of aflatoxin and glucomannan on some coagulation parameters. In the study, 40 New Zealand rabbits were used and the rabbits were separated equally into four groups as control (C), glucomannan (G), glucomannan + aflatoxin (AG) and aflatoxin (A). At the end of the (10 week) experiment, APTT and PT values in group A were significantly ($P < 0.05$) shorter than those in the control group. Fibrinogen concentrations were higher in groups A and AG than in the control group, but there was no significant difference between the groups. The thrombocyte count was significantly ($P < 0.05$) increased in group A compared with the other groups. In conclusion, in this study this aflatoxin dose did not cause coagulation disorders as seen by shortness in PT and APTT levels. This might be explained by the fact that this dose stimulated the coagulation mechanism due to the slight negatively effect which initiated coagulation response.

Key words: aflatoxin, glucomannan, rabbit, coagulation parameters

Introduction

One of the most important problem occurring as a result of unsuitable storage of food and foodstuff is toxication caused by mycotoxins produced by mould (ÇELİK et al., 2000a). Aflatoxins are the mostly seen mycotoxins. Aflatoxin B₁, which is produced by *Aspergillus flavus* and *Aspergillus parasiticus*, is the most harmful (ÇELİK et al., 1996; ERASLAN et al., 2004; ABDEL-WAHAB et al., 2002). Susceptibility to the toxic effects of aflatoxin B₁ varies between species. Rabbits are amongst most sensitive animal species (BAKER and GREEN, 1987). Aflatoxicosis results in with anaemia (KEÇEÇI et al., 1998), inhibition of immune function (ÇELİK et al., 2000b), harmful effects in the liver and kidneys (JINDAL et al., 1994), mutagenesis, teratogenesis, carcinogenesis and haemorrhages (ŞEHU et al., 2005).

Significant changes in serum biochemical and haematological parameters are seen in aflatoxicosis cases and these can assist in the diagnosis of toxications (BASMACIOĞLU

*Corresponding author:

Dr. Nurcan Dönmez, Selcuk University, Veterinary Faculty, Department of Physiology, Konya, Turkey, E-mail: nurcandonmez@selcuk.edu.tr

et al., 2005). Clinical bleeding and abnormal coagulation have been noted in animals intoxicated by aflatoxin B1 both experimentally and naturally. KEÇECI et al. (1995) have reported that aflatoxicosis caused a decrease in haemoglobin, haematocrit values and thrombocyte counts in broilers.

Removing AF from contaminated food and foodstuffs remains a major problem and there is a great demand for effective decontamination technology. Decontamination procedures have focused on degrading, destroying, inactivating or removing AF by physical, chemical or biological methods (OĞUZ et al., 2000). The beneficial effects of *Saccharomyces cerevisiae* (SCE) have been attributed to mannan which is a cell wall component (DIAZ et al., 2002; RAJU and DEVEGOWDA, 2000). Esterified glucomannan (EG) showed very high binding ability (80-97%) with AF (DIAZ et al., 2002; BASMACIOĞLU et al., 2005). The studies performed with EG at different concentrations of AF showed that EG partially or completely reversed the effect of AF on performance, biochemistry, haematology and immune response (RAJU and DEVEGOWDA, 2000; ARAVIND et al., 2003; SANTIN et al., 2003).

The aim of this study was to evaluate coagulation abnormality in rabbits intoxicated with aflatoxin to determine the counteraction of glucomannan application to aflatoxicosis.

Materials and methods

In the present study, 40 healthy New Zealand white rabbits were used. The rabbits were divided into 4 equal groups where the weight of each group of animals was close to each other. All the rabbits were kept in individual cages during the experiment (10 weeks) and were fed ad libitum as follows:

Group 1 (C); fed with pellet food

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

Group 3 (AG); fed with pellet food containing 1000 ppm glucomannan + 125 ppb aflatoxin

Group 4 (G); fed with pellet food containing 1000 ppm glucomannan

During the experiment animals had free access to water.

At the end of the experiment, blood samples were collected by cardiac puncture into citrated tubes to evaluate coagulation parameters. Thrombocyte counts were determined by haemocytometer. PT (prothrombin time), APTT (partial thromboplastin time) and fibrinogen determination was determined by commercial kits (DiaMed, DiaFibrinogen B305100; DiaCelin Liquid Cephaloplastin B301100; DiaMed, DiaPlastin B300240) using a coagulometer (DiaLab, Diaclot, C4Combi Coagulometer).

Statistical differences among the groups were tested by Duncan's multiple range test using SPSS for Windows, version 10.0. $P < 0.05$ was considered significant.

Results

Comparison of some coagulation parameters of the control and treatment groups is shown in Table 1.

Table 1. Coagulation parameters in control and aflatoxin and glucomannan groups.

Parameters	C	G	AG	A
Thrombocyte ($\times 10^5/\text{mm}^3$)	402.40 \pm 0.43 ^c	405 \pm 0.10 ^c	417.66 \pm 0.33 ^b	488.6 \pm 0.28 ^a
PT (s)	9.45 \pm 0.47 ^a	7.91 \pm 0.21 ^b	7.33 \pm 0.48 ^{bc}	6.51 \pm 0.25 ^c
APTT (s)	19.20 \pm 0.60 ^a	17.70 \pm 0.79 ^{ab}	16.56 \pm 1.30 ^{ab}	15.80 \pm 0.61 ^b
Fibrinogen (mg/dL)	426.5 \pm 39.32	431.8 \pm 79.45	455.8 \pm 49.25	550.0 \pm 59.72

APTT: activated partial thromboplastin time; PT: prothrombin time; a, b, c: $P < 0.05$.

Discussion

The liver plays an important role in haemostasis. Hepatocytes synthesize large numbers of coagulation factors. Assessment of coagulation status is important in animals suspected of having liver disease, because altered haemostasis can contribute to clinical signs and complicate invasive diagnostic procedures. The commonly used tests to assess coagulation status include determination of prothrombin time (PT), activated partial thromboplastin (APTT) and fibrinogen (ÇAM et al., 2006). For this reason the effects of aflatoxin which has mitogenic, hepatotoxic, hepatocarcinogenic and immunosuppressive effects (ÇELİK et al., 2000a), were used to determine some coagulation parameters in rabbits.

CLARK et al. (1986) reported that the coagulation defect in rabbits given AFB1 was characterized by prolonged PTs and APTTs and decreased activity of fibrinogen and increased platelet numbers. Additionally OSUNA and EDDS (1982) found significantly increased PT and APTT in pigs fed aflatoxin. Also in another study, aflatoxicosis decreased factors V, VII and VIII activity, fibrinogen concentration, platelet number and detectable plasma fibrin monomers (BAKER and GREEN, 1987). ESPADA et al. (1997) reported that mycotoxicosis increased fibrinogen concentration and decreased PT values in broilers. Additionally some researchers have found decreased thrombocyte count in aflatoxin treatment groups (KEÇECİ et al., 1998; OĞUZ et al., 2000; BASMACIOĞLU et al., 2005).

In the present study, APTT and PT values in group A were significantly ($P < 0.05$) shorter than those in the control group. Moreover, fibrinogen concentrations were higher in groups A and AG than in the control group, but there was no significant difference between the groups. The thrombocyte count was also significantly ($P < 0.05$) increased in

group A compared with the other groups. The decreased PT and APTT values observed may be consistent with increased plasma fibrinogen concentration. At the same time the increase in thrombocyte and fibrinogen levels may demonstrate a regenerative response with a haemorrhagic tendency. These findings agreed with the above reports that explain the suppressive effects of AF on coagulation. Single additions of glucomannan to AF - free diets did not produce any negative changes compared to the control. This also supported the notion that glucomannan was inert and non-toxic in terms of coagulation parameters. Our results showed the beneficial effects of glucomannan in the detoxification of AF. The levels of the AG group close to the other groups showed the beneficial effects of glucomannan on aflatoxin.

In conclusion, although the coagulation defects of aflatoxicosis are primarily due to the diminished hepatic synthesis of coagulation and initiating intravascular coagulation and consumption of coagulation factors (BAKER and GREEN, 1987), in this study this aflatoxin dose did not cause coagulation disorders as seen in shortness in PT and APTT levels. This might be explained by the fact that this dose stimulated the coagulation mechanism due to a slight negative effect which initiates the coagulation response.

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References

- ABDEL-WAHHAB, M. A., S. A. NADA, F. A. KHALIL (2002): Physiological and toxicological responses in rats fed aflatoxin- contaminated diet with or without sorbent materials. *Animal Feed Sci. Tech.* 97, 209-219.
- ARAWIND, K. L., V. S. PATIL, G. DEVEGOWDA, B. UMAKANTHA, S. P. GANPULE (2003): Efficacy of esterified glucomannan to counteract mycotoxicosis in naturally contaminated feed on performance and serum biochemical, haematological parameters in broilers. *Poultry Sci.* 82, 571-576.
- BAKER, D. C., R. A. GREEN (1987): Coagulation defects of aflatoxin intoxicated rabbits. *Vet. Pathol.* 24, 62-70.
- BASMACIOĞLU, H., H. OĞUZ, M. ERGUL, R. ÇÖL, Y. O. BIRDANE (2005): Effect of dietary esterified glucomannan on performance, serum biochemistry and haematology in broilers exposed to aflatoxin. *Czech. J. Anim. Sci.* 50, 31-39.
- CLARK, J. D., C. E. GREENE, J. P. CALPIN, R. C. HATCH, A. V. JAIN (1986): Induced aflatoxicosis in rabbits: blood coagulation defects. *Toxicol. Appl. Pharmacol.* 86, 353-361.
- ÇAM, Y., E. ÇETIN, A. İÇA, Ö. ATALAY, N. ÇETIN (2006): Evaluation of some coagulation parameters in hepatic coccidiosis experimentally induced with *Eimeria stiedae* in rabbits. *J. Vet. Med.* 53, 201-202.

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- ÇELİK, İ., Ö. DEMET, H. H. DÖNMEZ, H. OĞUZ, M. BOYDAK (1996): Determination of phagocytic and candidacidal activities of peritoneal macrophages isolated from chickens fed with aflatoxin and an aflatoxin adsorbing agent, polyvinyl polypyrrolidone (PVPP) containing feed. *Vet. Bil. Derg.* 12, 145-151.
- ÇELİK, İ., H. OĞUZ, Ö. DEMET, M. BOYDAK, H. H. DÖNMEZ, E. SUR, F. NIZAMLIOĞLU (2000a): Embryotoxicity assay of aflatoxin produced by *Aspergillus parasiticus* NRRL 2999. *Br. Poult. Sci.* 41, 401-409.
- ÇELİK, İ., H. OĞUZ, Ö. DEMET, H. H. DÖNMEZ, M. BOYDAK, E. SUR (2000b): Efficacy of polyvinylpolypyrrolidone in reducing the immunotoxicity of aflatoxin in growing broilers. *Br. Poult. Sci.* 41, 430-439.
- DIAZ, D. E., W. M. HAGLER, B. A. HOPKINS, L. W. WHITLOW (2002): Aflatoxin Binders I: *In vitro* binding assay for aflatoxin B1 by several potential sequestering agents. *Mycopathologia* 156, 223-226.
- ERASLAN, G., B. C. LIMAN, B. K. GÜÇLÜ, A. ATASEVER, A. N. KOÇ, L. BEYAZ (2004): Evaluation of aflatoxin toxicity in Japanese quails given various doses of hydrated sodium calcium aluminosilicate. *Bull. Vet. Inst. Pullawy* 48, 511-517.
- ESPADA, Y., R. RUIZ DE GOPEGUI, C. CUADRAS, F. J. CABANES (1997): Fumosis mycotoxicosis in broilers: plasma proteins and coagulation modifications. *Avian Dis.* 41, 73-79.
- JINDAL, N., S. K. MAHPAL, N. K. MAHAJAN (1994): Toxicity of aflatoxin B1 in broiler chicks and its reduction by activated charcoal. *Res. Vet. Sci.* 56, 37-40.
- KEÇECİ, T., Ö. DEMET, H. OĞUZ (1995): Single and combination effects of dietary aflatoxin and adsorbent (Mycifix Plus) on some hematological and serum biochemical parameters of broiler chickens. *J. Vet. Sci.* 11, 95-101.
- KEÇECİ, T., H. OĞUZ, V. KURTOĞLU, Ö. DEMET (1998): Effects of polyvinylpolyprolidone, synthetic zeolite and bentonite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. *Br. Poult. Sci.* 39, 452-458.
- OĞUZ, H., T. KEÇECİ, Y. O. BIRDANE, F. ÖNDER, V. KURTOĞLU (2000): Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. *Res. Vet. Sci.* 69, 89-93.
- OSUNA, O., G. T. EDDS (1982): Toxicology of aflatoxin B1, warfarin and cadmium in young pigs: clinical chemistry and blood coagulation. *Am. J. Vet. Res.* 43, 1387-1394.
- RAJU, M. V. L. N., G. DEVEGOWDA (2000): Influence of esterified glucomannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). *Br. Poult. Sci.* 41, 640-650.
- SANTIN, E., A. C. PAULILLO, A. MAIORKA, L. S. O. NAKAGHI, M. MACARI, A. V. F. SILVA, A. C. ALESSI (2003): Evaluation of the efficacy of *Saccharomyces Cerevisiae* cell wall to ameliorate the toxic effects of aflatoxin in broilers. *Int. J. Poultry Sci.* 2, 341-344.

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ŞEHU, A., S. ÇAKIR, Ö. CENGİZ, D. EŞSİZ (2005): Mycotox and aflatoxicosis in quails. Br. Poult. Sci. 46, 520-524.

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SAŽETAK

Cilj istraživanja bio je procijeniti učinke aflatoksina i glukomanana na neke pokazatelje grušanja. Istraživanje je provedeno na 40 novozelandskih kunića podijeljenih u četiri jednake skupine: kontrolnu skupinu, skupinu koja je dobivala glukomanan, skupinu koja je dobivala istodobno aflatoxin i glukomanan te skupinu koja je dobivala samo aflatoxin. Na kraju pokusa koji je trajao 10 tjedana aktivirano parcijalno tromboplastinsko vrijeme (APTT) i protrombinsko vrijeme (PT) bilo je značajno kraće ($P < 0,05$) u skupini koja je dobivala aflatoxin nego u životinja kontrolne skupine. Koncentracije fibrinogena bile su veće u kunića skupine koja je dobivala aflatoxin i skupine koja je dobivala istodobno aflatoxin i glukomanan nego u kontrolne skupine, ali nije bila ustanovljena statistički značajna razlika između skupina. Broj trombocita bio je značajno ($P < 0,05$) veći u kunića skupine koja je dobivala aflatoxin u usporedbi s drugim skupinama. Zaključuje se da davane doze aflatoksina nisu uzrokovale poremećaje grušanja kako se vidi iz PT i APTT. To se može objasniti time da je davana doza potaknula mehanizam grušanja zbog blagoga negativnoga učinka koji je bio začetnik grušanja.

Ključne riječi: aflatoxin, glukomanan, kunić, pokazatelji grušanja
