Effect of conjugated linoleic acid (CLA) on the performance and serum variables of broiler chickens intoxicated with aflatoxin B_1

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Abstract

The purpose of this study was to investigate the protective effect of dietary conjugated linoleic acid (CLA) on the prevention of aflatoxin B_1 (AFB_1) toxicity in the broiler chicken. A total of 99 Ross 308 male broiler chicks was assigned to nine treatments for 42 d. The experiment had a 3 X 3 factorial arrangement of treatments involving 0, 2 and 4 g CLA/kg feed, and 0, 200 and 300 ng AFB_1/kg feed. The parameters evaluated, were feed intake, body weight gain, feed efficiency, mortality, relative weights of liver and serum levels of total protein, total albumin, uric acid, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Aflatoxin B_1 in the diet negatively affected body weight gain and feed efficiency parameters when the birds were 21 and 42 days of age. Altered serum biochemical or hematologic measurements induced by AFB_1 treatments included increased serum activities of ALP, AST and ALT, decreased serum concentrations of albumin, total protein and uric acid. Liver weight was increased in birds given AFB_1 contaminated diet. The results indicated that CLA alone had insignificant effects on all parameters tested. However, birds receiving CLA+AFB_1 had a significantly higher body weight gain on day 42 than groups receiving AFB_1 alone. In addition, co-treatment with AFB_1 and CLA resulted in a significant improvement in feed efficiency and decreased relative weight of liver as compared with the AFB_1 groups. These results suggest that CLA provided protection against negative effects on liver damage induced by AFB_1 in broiler chickens.

Keywords: Aflatoxin B_1, conjugated linoleic acid, performance, biochemical parameters, broilers
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Introduction

The aflatoxins are a group of secondary metabolites produced by certain strains of fungi, e.g. *Aspergillus flavus* and *Aspergillus parasiticus* species. Agricultural commodities such as maize, peanut, cottonseed, wheat and other animal feedstuffs are frequently contaminated with aflatoxins. There are four major naturally occurring aflatoxins. The most hepatotoxic one is aflatoxin B_1 (AFB_1). Other structurally similar compounds are aflatoxins B_2, G_1 and G_2. All four have been detected as contaminants of crops before harvest, between harvesting and storing and after processing and manufacturing. In view of their potential hazards to human and animal health authorities in nearly all the countries of the world have imposed strict regulatory upper limits for aflatoxin concentrations in foods and feeds, usually at very low concentrations.

The main biological effects of aflatoxins are carcinogenicity, immunosuppression, mutagenity and teratogenity in a wide range of animals (Busby & Wogan, 1984). Avoidance of contaminated feed is rarely possible and feeds that contain relatively low concentrations of AFB_1 may still have deleterious effects on sensitive species, such as poultry (Doerr *et al.*, 1983; Giambrone *et al.*, 1985;).

Aflatoxins are metabolised by the liver and metabolic alterations are produced with a decrease in the synthesis of proteins, lipids, nucleic acids and vitamins (Ramos *et al.*, 1997). In poultry even small amounts of AFB_1 can cause a reduction in growth performance, hatchability and an increased susceptibility to bacterial and viral diseases and severe hepatotoxicosis (Kubena *et al.*, 1995; Verma *et al.*, 2003). These alterations produce changes in the biochemical parameters, mainly in enzyme levels as have been well documented in broiler chickens (Rosa *et al.*, 2001; Perozo *et al.*, 2003), turkeys (Giambrone *et al.*, 1985; Klein *et al.*, 2002), quails (Rao *et al.*, 1995; Oliveira *et al.*, 2002) and ducks (Cheng *et al.*, 2000). In serum, several clinical indicators are adversely affected by contaminated diet. They include serum urea, albumin, total protein, aspartate aminotransferase (AST, EC: 2.6.1.1), alanine aminotransferase (ALT, EC: 2.6.1.2) and gamma-glutamiltransferase (GGT, EC: 3.2.3.1) (Abdel-Wahhab *et al.*, 2002).

A variety of physical, chemical and biological approaches employed to counteract the aflatoxin problem has been reported in the literature on mycotoxins (Doyle *et al.,* 1982; Amra *et al.,* 1997). Dietary additions of zeolite and bentonite (Kececi *et al.,* 1998), hydrated sodium calcium aluminosilicate and activated charcoal (HSCAS) (Edrington *et al.,* 1996; Ramos & Hernandez, 1997), clinoptilolite (Oguz *et al.* 2000), dry yeast *Saccharomyces cerevisiae* (Celik *et al.,* 2001) and charcoal (Dalvi & Ademoyero, 1984; Dalvi & McGowan, 1984) have been used.

Conjugated linoleic acid (CLA) is a group of positional and geometrical isomers of linoleic acid C18:2 cis-9, cis-12. The CLA cis-9, trans-11 involves the exchange of the cis or trans configuration double bond in linoleic acid located positions 9 and 11 or 10 and 12. Conjugated linoleic acid has been shown to have anticarcinogenic effects in various cancer models, such as chemically induced skin, stomach and colorectal cancer (Aro *et al.,* 2000) and mammary tumorigenesis. Rats and mice that were fed CLA showed a lower tumour incidence and less tumour progression, and also reduced metastasis (Yurawecz *et al.,* 1999). In experiments with human breast cancer cell lines, CLA containing culture media decreased cell growth (Durgam & Fernandes, 1997). A recent study using CLA enriched butterfat, which contained the natural isomer distribution also showed anticarcinogenic effects (Aro *et al.,* 2000). Other beneficial effects of CLA include reduction in body fat, immuno-modulation and antioxidant properties (Cook *et al.,* 1993; Cantwell *et al.,* 1999).

Due to these properties CLA was selected for use in this experiment. The purpose of this experiment was to investigate the potential of CLA to reduce the toxic effects of AFB_1 with respect to growth performance, immune responses and other parameters in growing chickens.

Materials and Methods

A total of 99 1-day old male Ross 308 broiler chickens with a mean weight 42.8 g was weighed, individually caged, numbered and divided into nine treatment groups of 11 birds per group. The nine treatments, arranged according to a 3 x 3 factorial design, consisted of three levels of AFB_1, *viz.* 0, 200 and 300 ng/kg feed and three levels of CLA, *viz.* 0, 2 and 4 g/kg feed. Pure crystalline AFB_1 was obtained from Sigma-Makor Chemical Corp., Jerusalem, Israel. The AFB_1 was weighed and dissolved under a hood in warm chloroform at 1 mg/10 mL. The AFB_1 solution in chloroform was then sprayed on a thin layer (<1 cm) of the basal diet. The treated feed was left overnight at room temperature for the solvent to evaporate and was then mixed into the basal diet to provide the desired levels of AFB_1/kg of diet. Conjugated linoleic acid was purchased from the Cognis Corporation, U.S.A. A starter diet was formulated according to the NRC (1994) recommendations to meet the nutrient requirements of broilers during their first 0-21 days, and a grower diet for the following 22 to 42 days. The composition of the basal diets is presented in Table 1. The diets were analysed for aflatoxin content using thin layer chromatography (Howel, 1983).

Each experimental group of the birds received its specific diet *ad libitum*. Water was provided in continuous flow water troughs. The chicks were reared under a conventional temperature regimen, i.e. starting at 33 ^{o}C, and reduced by 3 ^{o}C/week to 21 ^{o}C. The relative humidity was maintained at between 60 and 70%. The birds were exposed to continuous lighting. Body weight gain, feed consumption and feed efficiency were calculated weekly. Mortality was recorded and the percentage calculated at the end of the study.

After 42 days all chickens were slaughtered by dislocation of the neck vertebrae and bleeding, and prepared for further analysis. Livers were removed and their weights recorded. Blood samples were collected from all birds from the retroorbital venous plexus at the end of the experimental period for haematological and biochemical study. Within one hour of collection the serum was separated. The serum was then analysed for alkaline phosphatase (ALP, EC: 3.1.3.1), ALT and AST activity, using the SNA-12 clinical method (Anonymous, 1974). Serum albumin concentration was measured using the bromocresol green binding assay (Doumas *et al.,* 1971), total protein in serum using the Biuret reaction (Doumas *et al.,* 1981) and serum uric acid concentration by enzymatic-colorimetry (Barham *et al.,* 1972).

All data were subjected to analysis of variance using the statistical analyses system, SPSS (1993). If appropriate, post-hoc analyses were carried out using the Duncan's test for multiple comparisons. Statements of statistical significance are based on $P < 0.05$.

Table 1 Composition of basal diets during the experiment (g/kg)

	0 – 21 days	22 – 42 days
Ingredients		
Maize	615.0	685.5
Soyabean meal	245.0	217.0
Fish meal	120.0	70.0
Dicalcium phosphate	5.1	12.0
DL-methionine	2.0	0.5
Salt	3.5	3.5
Vitamin premix[a]	3.5	3.5
Mineral premix[b]	2.5	3.5
Lysine	1.2	1.0
Choline-Cl	2.2	3.5
Total	1000	1000
Calculate analysis		
Crude protein	225	200
ME (MJ/kg)	12.88	13.38
Calcium	9.0	9.2
Phosphorus	7.4	7.2
L-lysine	12.0	10.0
Methionine+cystine	9.5	7.5

[a]Provided per kg of diet: Vitamin A - 8000 IU; vitamin D_3 - 1200 IU; vitamin E - 10 IU; vitamin K_3 - 2 mg; thiamine - 2 mg; riboflavin - 5 mg; pyroxidine - 0.2 mg; vitamin B_{12} - 0.03 mg; pantothenic acid - 10 mg; niacin - 50 mg; biotin - 0.1 mg; folic acid - 0.5 mg

[b]Provided per kg of diet: Iron - 80 mg; zinc - 40 mg; manganese - 60 mg; iodine - 0.8 mg; copper - 8 mg; selenium - 0.2 mg; cobalt - 0.4 mg

Results

Data presented in Table 2 show the effect of dietary AFB_1, CLA and their combinations on the growth performance of broilers from 0 to 21 days and 0 to 42 days of the experiment. There were no significant differences between treatment groups at the end of the first 21-day period. However, at the end of the experiment the results showed that the feed containing AFB_1 at 200 and 300 ng/kg feed caused decreases ($P < 0.05$) in body weight gain and feed efficiency as compared with the control group. The addition of CLA to the AFB_1 containing diets partially ameliorated the adverse effects of AFB_1 on body weight gain and feed efficiency. No statistically significant differences were found for the feed consumption of the broilers on different treatments. For the duration of the experiment, there was no mortality in any of the treatment groups.

Results of the serum biochemical analysis (Table 3) revealed that treatments with AFB_1 alone caused an increase ($P < 0.05$) in the serum activities of ALT, AST and ALP. Conjugated linoleic acid at 2 or 4 g/kg did not cause significant changes in these serum parameters as compared to the control. Moreover, chicks treated with AFB_1 plus CLA generally exhibited improved haematological parameters, but most of them were still higher than in the controls. Data presented in Table 3 show the effect of the dietary treatments on liver weights. The weights of the livers were significantly increased in chicks treated with AFB_1. Supplementation of diets containing AFB_1 with 2 or 4 g/kg CLA decreased the effect of the toxin on the liver weight. However, the addition of the CLA to the control diet had no effect on liver weight.

Discussions

Aflatoxins are very important to the poultry industry because of their toxicity and frequent occurrence in feedstuffs (Huff *et al.*, 1992). Chronic aflatoxicosis may be diagnosed by determining the serum biochemical and haematological alterations before clinical symptoms become apparent (Kececi *et al*., 1998). The use of adsorbent materials against mycotoxicosis is a new field that should be completely explored (Ramos & Hernandez, 1997). In contrast, CLA can have chemo-protective effects in several tissues during multiple stages of carcinogenesis (Belury, 1995) as well as immune enhancing qualities (Cook *et al.*, 1993) and possible antiatherogenic properties (Lee *et al.*, 1994).

Table 2 Effects of conjugated linoleic acid (CLA) and aflatoxin (AFB_1) on body weight gain (BWG), feed consumption (FC) and feed efficiency in male broiler from 0 to 21 and 0 to 42 days of age

Treatments	Body weight gain g/bird		Feed consumption g/bird		Feed efficiency FC/BWG	
	0-21 days	0-42 days	0-21 days	0-42 days	0-21 days	0-42 days
Control	693 ^{c}	2265 ^{bc}	892	3964	1.29 ^{a}	1.75 ^{a}
AFB_1 200ng/kg	613 ^{ab}	2091 ^{a}	803	3763	1.31 ^{ab}	1.80 ^{bc}
AFB_1 300 ng/kg	603 ^{a}	2025 ^{a}	796	3705	1.32 ^{b}	1.83 ^{d}
CLA 2 g/kg	719 ^{c}	2287 ^{c}	930	4024	1.29 ^{a}	1.76 ^{a}
CLA 4 g/kg	676 ^{abc}	2271 ^{bc}	881	3975	1.30 ^{ab}	1.75 ^{a}
AFB_1 200 ng/kg + CLA 2 g/kg	665 ^{abc}	2213 ^{bc}	862	3960	1.30 ^{ab}	1.79 ^{bc}
AFB_1 200 ng/kg + CLA 4 g/kg	656 ^{abc}	2245 ^{bc}	865	3996	1.32 ^{b}	1.78 ^{b}
AFB_1 300 ng/kg + CLA 2 g/kg	650 ^{abc}	2181 ^{bc}	850	3861	1.31 ^{ab}	1.77 ^{ab}
AFB_1 300 ng/kg + CLA 4 g/kg	640^{abc}	2181 ^{bc}	844	3901	1.32 ^{b}	1.79 ^{bc}
s.e.m.	9.32	20.06	13.2	70.2	0.006	0.01

Pooled s.e.m. - pooled standard error of the mean
^{a,b,c,d}Means within column with different superscripts differ significantly $P < 0.05$

Table 3 Effects of conjugated linoleic acid (CLA) on serum biochemical variables and liver weight in male broiler chickens receiving a diet containing aflatoxin (AFB_1) for 42 days

Treatments	ALP U/L	AST U/L	ALT U/L	Total protein g/dL	Albumin g/dL	Uric acid mg/dL	Liver weight g/bird
Control	556.9^{a}	235.8^{a}	3.8^{a}	4.16^{c}	1.44	4.36^{b}	44.8^{a}
AFB_1 200ng/kg	729.7^{cd}	275.2^{b}	5.1^{b}	3.60^{ab}	1.38	3.10^{a}	51.5^{bc}
AFB_1 300 ng/kg	791.2^{c}	277.9^{b}	5.8^{b}	3.30^{a}	1.36	2.94^{a}	53.4^{c}
CLA 2 g/kg	605.0^{ab}	233.0^{a}	3.6^{a}	4.18^{c}	1.48	4.40^{b}	45.2^{a}
CLA 4 g/kg	595.6^{ab}	243.4^{a}	3.4^{a}	4.02^{bc}	1.42	4.28^{b}	44.8^{a}
AFB_1 200 ng/kg + CLA 2 g/kg	671.7^{bc}	249.1^{a}	4.0^{ab}	3.96^{bc}	1.40	4.04^{ab}	46.1^{a}
AFB_1 200 ng/kg + CLA 4 g/kg	634.5^{abc}	253.0^{ab}	4.2^{ab}	4.06^{c}	1.42	3.80^{ab}	45.5^{a}
AFB_1 300 ng/kg + CLA 2 g/kg	709.7^{bc}	261.2^{ab}	4.9^{b}	3.94^{bc}	1.37	3.56^{a}	47.9^{ab}
AFB_1 300 ng/kg + CLA 4 g/kg	628.7^{abc}	255.6^{ab}	4.4^{ab}	3.92^{bc}	1.41	3.62^{ab}	45.9^{a}
s.e.m.	13.44	3.73	0.06	0.09	0.01	0.05	0.88

ALP – alkaline phosphatase; AST - aspartate aminotransferase; ALT - alanine aminotransferase
Pooled s.e.m. - pooled standard error of the mean
^{a,b,c,d}Means in column with different superscripts differ significantly $P < 0.05$

In this study the results showed that the inclusion of AFB_1 at 200 or 300 ng/kg in the diet significantly reduced growth parameters of broiler chickens such as body weight gain and feed efficiency at the end of the 42-day feeding period. These results agreed with the findings of Rosa *et al.* (2001) and Bailey *et al.* (1998). Kubena *et al.* (1997) also reported that AFB_1 has a negative effect on body weight gain and feed efficiency in broiler chickens. Addition of CLA to the AFB_1 containing diet significantly alleviated the adverse effects of AFB_1 on body weight gain and feed efficiency.

The activities of ALT and AST in serum are sensitive indicators of acute hepatic necrosis, and ALP level is known to be indicative of hepatobiliary disease (Kaplan, 1987). The biochemical changes and alterations in enzyme activities induced a stress on liver function (Abdel-Wahhab *et al.,* 1999). The present study showed that broiler chickens fed an AFB_1-contaminated diet had a significant increase in serum ALT, AST and ALP activities compared to those of the controls. This is a well-known effect of aflatoxicosis (Huff *et al.,* 1986). The literature on the effect of adding bentonite to the diet to prevent the adverse effect of aflatoxin is equivocal. Kececi *et al.* (1998) reported that some serum biochemical changes could be ameliorated by the addition of bentonite at doses of 5 mg/kg in broiler chicken diets. On the other hand, Santurio *et al*. (1999) observed that sodium bentonite did not alter the aflatoxin induced changes in the biochemistry of broilers significantly. Although the activities of these enzymes in the present study were reduced in the diets containing CLA + AFB_1, they did not reach the normal values of the control, even when the higher level of CLA, 4 g/kg, was used. Thus, the properties of CLA in regulating physiological and metabolic responses to immunological challenges remain to be elucidated. Kubena *et al.* (1990) demonstrated that when hydrated sodium calcium aluminosilicate (HSCAS) was added to broiler chicken diets contaminated with 5 mg aflatoxin, toxicity was reduced. However, it did not effect serum biochemical parameters such as AST activity or serum total protein and albumin concentrations.

Decreases in serum total protein concentration are frequently observed in chickens suffering from aflatoxicosis (Kubena *et al.,* 1997; Kececi *et al.,* 1998). The inhibition of protein synthesis in the liver and the decrease of serum protein concentrations during aflatoxicosis have been reported (Jindal *et al.,* 1994). In the present study, serum total protein and uric acid concentrations were reduced in the treatments containing AFB_1, These effects were ameliorated by the addition of 4 g CLA/kg to the diets containing AFB_1. The beneficial effect of CLA might be related to the fact that CLA feeding induced a rapid and marked decrease in fat accumulation and an increase in protein deposition (DeLany *et al*., 1999). Moreover, Cook *et al.* (1993) observed that 0.5% dietary CLA modified some aspects of the immune response in chicks.

The liver is considered the principal target organ for aflatoxin toxicity and one classic symptom of aflatoxicosis in broiler is an increase in liver weight (Miazzo *et al*., 2000). In the present study AFB_1 at both the 200 and 300 ng/kg levels of inclusion caused significant increases in liver weight. The addition of CLA reduced the magnitude of these increases, thus indicating a direct protective effect of CLA on the liver.

The present experiment showed that broiler chicks consuming an aflatoxin contaminated diet experienced a significant decrease in body weight gain with a poorer feed efficiency and increases in activities of serum enzymes. The addition of CLA to the aflatoxin-contaminated diet could reduce the negative effect of aflatoxin on growth performance and liver weight of the broilers.

Conclusions

Our findings suggest that adding CLA to broiler diets could partially protect the birds against some of the more extreme toxic effects of AFB_1. This protective action was evident in body weight gains, feed efficiency and liver weights. These data suggest that CLA could be used to prevent the adverse effects arising from AFB_1 ingestion. Further studies are required using different levels of CLA to determine optimal levels of CLA addition to minimise the adverse effects of aflatoxicosis on weight gain and liver functions.

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References

Abdel-Wahhab, M.A., Nada, S.A. & Khalik, F.A., 2002. Physiological and toxicological responses in rats fed aflatoxin-contaminated diet with or without sorbent materials. Anim. Feed Sci. Technol. 97, 209-219.

Amra, H.A., Abdel-Galil, M.M. & Abdel-Vahhab, M.A., 1997. Biological control of aflatoxins, ochratoxin A and/or fumonisin B production by Thtichoderma *harzianum* on corn. J. Agr. Sci. Mansoura Univ. 11(22), 3821-3829.

Anonymous, 1974. Technical publication No. UA4-0160-00, Technion Instr. Corp., Terrytown, 1974. pp. 127-129.

Aro, A.S., Mannisto, I., Salminen, M.L., Onaskainen, V., Kataja, V. & Uustupa, M., 2000. Inverse association between dietary and serum conjugated linoleic acid and risk of breast cancer in postmenopausal women. Nutr. Cancer 38, No. 2, 151-157.

Bailey, R.H., Kubena, L.F., Harvey, R.B., Buckley, S.A. & Rottinghaus, G.E., 1998. Efficacy of various inorganic sorbents to reduce the toxicity of aflatoxin and T-2 toxin in broiler chicksens. Poult. Sci. 77, 1623-1630.

Barham, D. & Trinder, P., 1972. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 97, 142-145.

Belury, M.A., 1995. Conjugated dienoic linoleate: a polyunsaturated fatty acid with unique chemoprotective properties. Nutr. Rev. 53, 83-89.

Busby, W.F. & Wogan, G.N., 1984. Aflatoxins. In: Chemical Carcinogens. Ed. Searle, C.E., American Chemical Society, Washington, DC. pp. 945–1136.

Cantwell, H.R., Devery, O'Shea, M. & Stanton, C., 1999. The effect of conjugated linoleic acid on the antioxidant enzyme defence system in rat hepatocytes. Lipids 34, 833-839.

Cheng, Y.H., Shen, T.F., Pang, V.F. & Chen, B.J., 2000. Effects of aflatoxin and carotenoids on growth performance and immune response in mule ducklings. Comp. Biochem. Phys. 128, 19-26.

Celik, K., Denli, M., Ertürk, M., Öztürkcan, O. & Doran, F., 2001. Evaluation of dry yeast *Saccharomyces Cerevisiae* in the feed to reduce aflatoxin B_1 AFB_1 residues and toxicity to Japonica Quails *Coturnix coturnix Japonica*. J. Appl. Anim. Res. 20, 245-250.

Cook, M.E., Miller, C.C., Park, Y. & Pariza, M.W., 1993. Immune modulation by altered nutrient metabolism: Nutritional control of immune-induced growth depression. Poult. Sci. 72, 1301-1305.

Dalvi, R.R. & Ademoyero, A.A., 1984. Toxic effects of aflatoxin B1 in chickens given feed contaminated with *Aspergillus flavus* and reduction of toxicity by activated charcoal and some chemical agents. Avian Dis. 28, 61-69.

Dalvi, R.R. & McGowan, C., 1984. Experimental induction of cronic aflatoxicosis in chickens by purified aflatoxin B1 and its reversal by activated charcoal, phenobarbital and reduced glutathione. Poult. Sci. 63, 485-491.

DeLany, J.P., Blohm, F., Truett, A.A., Scimeca, J.A. & West, D.B., 1999. Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. Am. J. Physiol. 276, 1172-1179.

Doerr, J.A., Huff, W.E., Wabeck, C.J., Chaloupka, G.W., May, J.D. & Merkley, J.W., 1983. Effects of low-level chronic aflatoxicosis in broiler chickens. Poult. Sci. 62, 1971–1977.

Doumas, B.T., Watson, W.A. & Biggs, H.G., 1971. Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chim. Acta. 31, 87-96.

Doumas, B.T., Bayse, D.D., Carter, R.J., Peters, J.T. & Schaffer, R.A., 1981. Candidate reference method for determination of total protein in serum. I. Development and Validation, II. Tests for transferability. Clin. Chem. 27, 1642-1654.

Doyle, M.P., Applebaum, R.S., Brackett, R.E. & Marth, E.H., 1982. Physical, chemical and biological degradation of mycotoxins in food and agricultural commodities. J. Food Prot. 45, 964-971.

Durgam, R.V. & Fernandes, G., 1997. The growth inhibitory effect of conjugated linoleic acid on MCF-7 cells is related to estrogen response system. Cancer Lett. 116, 121–130.

Edrington, T.S., Sarr, A.B., Kubena, L.F., Harvey, R.B. & Phillips, T.D., 1996. Hydrated sodium calcium aluminosilicate HSCAS, acidic HSCAS, and activated charcoal reduce urinary excretion of aflatoxin M1 in turkey poults. Lack of effect by activated charcoal on aflatoxicosis. Toxicol. Lett. 1999, 89, 115-122.

Giambrone, J.J., Diener, U.L., Davis, N.D., Panangala, V.S. & Hoerr, F.J., 1985. Effects of aflatoxin on young turkeys and broiler chickens. Poult. Sci. 64, 1678-1684.

Howel, M.V., 1983. Methods for determination of aflatoxins, ochratoxin A and zearalenone in mixed feeds with detection by thin layer chromatography or high performance liquid chromatography. Proc. Int. Symp. Mycotoxins. National Research Centre. Cairo, Egypt, 1983. pp. 293-296.

Huff, W.E., Kubena, L.R., Harvey, R.B. & Phillips, T.D., 1992. Efficacy of hydrated sodium calcium aluminosilicate to reduce the individual and combined toxicity of aflatoxin and ochratoxin A. Poult. Sci. 71, 64-69.

Jindal, N., Mahipal, S.K. & Mahajan, N.K., 1994. Toxicity of aflatoxin B_1 in broiler chickens and its reduction by activated charcoal. Res. Vet. Sci. 56, 37-40.

Kaplan, M.M., 1987. Laboratory tests. In: Diseases of the Liver. Ed. Schiff, L. & Schiff, E.R., J.B. Lippincott, Philadelphia. p. 219.

Kececi, O., Oguz, H., Kurtoglu, V. & Demet, Ö., 1998. Effects of polyvinylpolypyrrolidone, synthetic zeolite and bentonite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Br. Poult. Sci. 39, 452-458.

Klein, P.J., Vleet, T.R.V., Hall, J.O. & Coulombe, R.A., 2002. Dietary butylated hydroxytoluene protects against aflatoxicosis in Turkey. Toxicol. Appl. Pharm. 182, 11-19.

Kubena, L.F., Harwey, R.B. & Huff, W.E., 1990. Efficacy of hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. Poult. Sci. 69, 1078-1086.

Kubena, L.F., Edrington, T.S., Kamps-Holtzapple, C., Harvey, R.B., Elissalde, M.H. & Rottinghaus, G.F., 1995. Effects of feeding fumonisin B_1 present in *Fusarium moniliforme* culture material and aflatoxin singly and in combination to turkey poults. Poult. Sci. 74, 1295-1303.

Kubena, L.F., Harvey, W.E., Buckley, S.A., Edrington, T.S. & Rottinghaus, G.E., 1997. Invidual and combined effects of moniliformin present in *Fusarium fujikuroi* culture material and aflatoxin in broiler chicks. Poult. Sci. 76, 265-270.

Lee, K.N., Kritchevsky, D. & Pariza, M.W., 1994. Conjugated linoleic acid and atheroscierosis in rabbits. Atheroscierosis 108, 19-25.

Miazzo, R., Rosa, C.A.R., De Queiroz Carvalho, E.C., Magnoli, C., Chiacchiera, S.M., Palacio, G., Saenz, M., Kikot, A., Basaldella, A. & Dalcero, A. 2000. Efficacy of synthetic zeolite to reduce the toxicity of aflatoxin in broiler chicks. Poult. Sci. 78, 1-6.

NRC, 1994. Nutrient requirements of poultry. (9th ed.). National Academy Press, Washington, D.C., USA.

Oliveira, C.A.F., Rosmaninho, J.F., Butkeraitis, P., Correa, B., Reis, T.A., Guerra, J.L., Albuquerque, S.R. & Moro, M.E.G., 2002. Effect of low levels of dietary aflatoxin B_1 on laying Japanese quail. Poult. Sci. 81, 976-980.

Oguz, H. & Kurtoglu, V., 2000. Effect of clinoptiloite on performance of broiler chickens during experimental aflatoxicosis. Br. Poult. Sci. 41, 512-517.

Ramos, A.J. & Hernandez, E., 1997. Prevention of aflatoxicosis in farm animals by means of hydrated sodium calcium aluminosilicate addition to feedstuff: a review. Anim. Feed Sci. Technol. 65, 197-206.

Rao, J.R., Sharma, N.N. & Johri, T.S., 1995. Influence of dietary aflatoxin on *Eimeria uzura* infection in Japanese quail. Vet. Parasitol. 56, 17-22.

Rosa, C.A.R., Miazzo, R., Magnoli, C., Salvano, M., Chiacchiera, S.M., Ferrero, S., Saenz, M., Carvalho, E.C.Q. & Dalcero, A., 2001. Evaluation of the efficacy bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers. Poult. Sci. 180, 139-144.

SPSS, 1993. SPSSx for Windows. Release, 6.0, Copyright: SPSS Inc., 1989-1993, New York, USA.

Verma, J., Johri, T.S. & Swain, B.K., 2003. Effect of varying levels of aflatoxin, ochratoxin and their combinations on the performance and egg quality characteristics in laying hens. Asian-Aust. J. Anim. 167, 1015-1019.

Yurawecz, M., Mossoba, M., Kramer, J., Pariza, M. & Nelson, G., 1999. Advances in conjugated linoleic acid research. Vol. 1. AOCS Press, Champaign, Illinois, USA.