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RESEARCH ARTICLE

Hepatoprotective Role of Milk Thistle (*Silybum marianum*) in Meat Type Chicken Fed Aflatoxin B_1 Contaminated Feed

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ABSTRACT

Milk thistle was added in aflatoxin B_1 contaminated poultry feed to investigate and compare its hepatoprotective effects with a commercial toxin binder. Two hundred and forty, day-old broilers were randomly allocated into four major groups A, B, C and D. Group A was kept as control, having aflatoxin free feed, while group B was fed aflatoxin contaminated feed, group C was raised on aflatoxin contaminated feed with toxin binder "Mycoad" @ 3g/kg of feed, while group D was provided aflatoxin contaminated feed along with milk thistle @10g/kg of feed. Aflatoxin B_1 was present at the level of 80 µg/kg feed during the first week and 520 µg/kg feed in the remaining experimental period. Serum total protein was significantly ($P<0.05$) higher in group D, followed by group A, C and B. Serum enzymes including, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values were significantly ($P<0.05$) lower in group D, followed by C, A and B, which are indicative of hepatoprotective role of milk thistle. Body weight gain and feed intake was decreased by aflatoxin contaminated feed (group B) in comparison with group A and group D. Milk thistle supplementation improved body weight gain and feed intake and was similar to toxin binder treated birds. Average feed conversion ratio (FCR) was significantly ($P<0.05$) higher (poor) in group B and were the same in all other groups. Present study demonstrated that milk thistle can potentially be used as mycotoxin binder and to minimize the adverse effects of toxin contaminated feed in broilers production.

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INTRODUCTION

Aflatoxin B_1 is the most prevalent form of aflatoxins (Ahsan *et al*., 2010) and as a general rule, growing poultry should not receive more than 20 ppb aflatoxin in the diet (Sabri *et al*., 1989). Poultry fed aflatoxin contaminated diet has depressed immunity and impaired liver function (Hussain *et al*., 2008; Hassan, 2010; Khan, 2010). Affected birds have been reported to have abnormal coloration, size and damaged liver (Denli and Okan, 2006). Liver inflammation (hepatitis) and immune disfunction have also been reported in the literature (Asim *et al*., 1990). The severity of adverse effects of aflatoxin in broilers depends on the level and exposure to contaminated diet. Infected livers exhibit macroscopic and microscopic lesions (Hussain *et al*., 2008).

Milk thistle, a medicinal herb found in Pakistan, has been extensively used in folk medicine for treating liver diseases. Certain active ingredients found in the seed of this plant possess numerous medicinal properties. Earlier in 1960, a German scientist isolated a flavonoid 'silymarin' from milk thistle (Simanek *et al*., 2000). Chemically it is composed of 4 flavonoids, silybin, isosilybin, silydianin and silychristin (Pradhan and Girish, 2006). Silybin is the major component constituting 50 to 70% of silymarin and exhibit greater biological activities (Ludovico *et al*., 2010). Pradhan and Girish (2006) reported that milk thistle has hepatoprotective and hepatorestorative functions and protects liver and kidney from both exo- and endo-toxins. It has reduced liver enzyme production and has shown anti-inflammatory and T cell-modulating effects (Janice *et al*., 2007). In ancient

times, milk thistle was used to treat hepatitis, cirrhosis, jaundice, snake bites, insect stings, mushroom poisoning, and alcohol (Abenavoli *et al*., 2011). Birds affected by aflatoxin B_1 can effectively be recovered if treated with milk thistle (Grizzle *et al*., 1999; Tedesco *et al.,* 2004; Kalorey *et al*., 2005). Major objective of this research study was to evaluate the efficacy of milk thistle in broilers fed aflatoxin B_1 contaminated feed and to compare it with a commercially available toxin binder.

MATERIALS AND METHODS

This research project was conducted at Poultry Unit of Khyber Pakhtunkhwa Agricultural University, Peshawar. All procedures involving birds were pre-approved by the University Board of Studies for welfare issues.

Bird Husbandry and Experimental Protocol: Total 240, day-old broilers were randomly allocated to four groups (A, B, C and D) in 4x 2 x 3 factorial experiment, each having two sub-groups (4x2=8). Birds in group A were kept as control i.e. received mycotoxins free diet, while that in group B, C and D were maintained on diet having (aflatoxin B_1) AFB_1, AFB_1 plus a commercial toxin binder Mycoad® (3 g kg^{-1} feed) and AFB_1 plus milk thistle (10 g kg^{-1} feed), respectively. AFB_1 was present at the level of 80 µg/kg feed during the first week and 520 µg/kg feed in the remaining experimental period. Half of the birds (sub-group) in each group were vaccinated against ND, IB and IBD and were replicated (n=3) with 10 chicks/replicate. This trial was conducted in an open sided house and birds were given free access to feed and water. Birds were consistently monitored and optimum environmental conditions were maintained inside the shed. The experiment was continued for 5 weeks.

Contaminating poultry feed: *Aspergillus flavus* was isolated from contaminated feed ingredients and cultured on Sabouraud agar at optimum conditions in the Veterinary Research Institute, Peshawar. This culture was then introduced to commercial feed placed in a humid and hotter region to fully propagate the toxin in feed. Representative samples of feed were analyzed for aflatoxin using thin layer chromatography (TLC) technique (Rahim *et al.*, 1999). Commercial feed was contaminated on weekly basis to maintain the toxin within the required limits.

Milk thistle and Mycoad®: The seeds of milk thistle (*Silybum marinum)* were collected from Warsak and adjoining tribal areas of Michini, Mohmand Agency. Dried seed was grinded and added to contaminated feed. A commercially available mycotoxin binder Mycoad® was also used for comparative study.

Serum Biochemical parameters: On the last day of the study two birds were randomly selected from each replicate to collect blood samples for liver function tests (LFTs). Blood samples were centrifuged at 4000 rpm for ten minutes and serum was stored. Liver function tests included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total serum proteins. Serum albumin was measured by the bromcrezol green method (Anonymous, 1984). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by the ultraviolet spectrometry method (Anonymous, 1984) using commercially available kits (AMP Diagnostic, Austria. BV 6050).

Statistical Analysis: Data were subjected to statistical analyses using standard procedures of analysis of variance (ANOVA). SAS (SAS, 1998) was used to run all data analyses.

RESULTS AND DISCUSSION

Total Serum Protein: Feeding AFB_1 contaminated diet to broiler chicks resulted in a significant decrease (2.35±0.08 [mean±SD]) in the serum total protein concentration (Table 1), compared to the values exhibited by the chicks in group A (4.96±0.09). This decrease in the values of serum total protein might be due to hepatotoxic effects of AFB_1, as it has earlier been reported by Denli and Okan (2006), Kalorey *et al*. (2005) and Tedsco *et al*. (2004). Feeding commercially available mycotoxins binder Mycoad gave partial protection against the aflatoxin-induced damage, by elevating the serum protein levels (4.70±0.07). However, a complete restoration of liver damage was shown by the milk thistle extract in group D, which showed a non significant difference in the values of serum total protein compared to the chicks in group A (Birds fed mycotoxins free diet). Although there is no comparable study in the literature describing the ameliorative effect of milk thistle extract upon the serum protein values, however this protective effect might be due to its antioxidant properties, which prevented the free radical-induced hepatocytes damage (Abenavoli *et al*., 2011). Sylimarin also promotes liver cell protein synthesis and decreases the oxidation of glutathione. A non significant effect was noted in the serum total protein concentration by vaccinating the chicks against different disease.

Alkaline Phosphatase (ALP): Alkaline phosphatase was significantly affected by treatments and non-significantly by vaccination. Interaction effects were also non-significant (Table 1). A significant higher value (671.56±15.62) of serum ALP was observed in the group fed AFB_1 alone as compared to the value noted in the chicks in group A (465.14±14.77). It is evident from the findings that AFB_1 caused liver damage and leakage of enzymes, resulting in elevated ALP levels. However, supplementation of broiler feed with mycotoxin binder and milk thistle extract prevented the rise in values of alkaline phosphatase by keeping liver healthy due to its antioxidative property. Similar results were reported by Pradhan and Girish (2006) and Janice *et al*. (2007), who reported reduced liver enzyme levels by milk thistle. Tedesco *et al*. (2004) also recorded reduction of AST, ALT and ALP by milk thistle in experimentally induced aflatoxicosis in broilers. Dhiman and Chawal (2005) also reported considerable prevention of serum enzymes leakage and consequently preventing rise of alkaline phosphatase by milk thistle.

Alanine aminotransferase (ALT): The values of serum ALT were significantly higher (64.66±1.76) in groups fed AFB1 contaminated ration as compare to the chicks in group A (48.33±3.41) or the chicks fed commercially

available mycotoxins binder (51.00±3.23) and milk thistle extract (46.00±3.64). These findings clearly showed the hepatoprotective effect of milk thistle extract (Table 1). Abenavoli *et al*. (2011) and Ludovico *et al*. (2010) reported similar hepatoprotective property of milk thistle which is in agreement with findings of the present study. Wang *et al*. (2010) also reported the hepatoprotective property of silibinin, which is the active ingredient of milk thistle. Kalorey *et al*. (2005) reported reduction of serum enzymes (AST, ALT and ALP) in aflatoxicosis in broiler chicks by medicinal herbs. Similarly Khan (2007) and Ghani (2007), also recorded reduction of ALT by supplementation of feed with medicinal herb in broilers. Thyagarajan *et al*. (2002) also reported that *Glycyrrhiza glabra* had been shown to be hepatoprotective and capable of inducing an indigenous interferon, which support our findings (Thyagarajan *et al.*, 2002)**.**

Aspartate aminotransferase (AST): Serum AST values were significantly affected by various treatments and vaccination. Combined effect of treatment and vaccination was non-significant (Table 1). Aflatoxin administration in the feed of broiler chicks caused severe damage to hepatocytes causing leakage of serum enzymes, which ultimately led to rise of AST in group B. Lowest AST values in group D (milk thistle fed group) are indicative of the hepatoprotective property of milk thistle. The findings of the present study are in close agreement to those of Dhiman and Chawal (2005), who reported reduction of ALT and AST by milk thistle in human hepatitis B and C patients. Our results are also supported by Khan (2007) and Ghani (2007), who recorded reduction of AST by supplementation of feed with medicinal herbs. Thyagarajan *et al.* (2002) also reported that *Glycyrrhiza glabra* had hepatoprotective effects and capable of inducing an indigenous interferon.

Performance of broiler chicks: Performance of broiler chicks was measured in terms of body weight gain, feed intake, and feed conversion ratio. Body weight gain and feed intake was depressed by aflatoxin contaminated feed (group B) (Table 2). Body weight gain and feed intake was improved by milk thistle supplementation (Group D) and was not different from the toxin binder treated birds (Group C).

Average feed conversion ratio (FCR) was significantly ($P< 0.05$) higher (poor) in group B and were the same in all other groups (Table 2). Findings of the present study are supported by Hassan (2010) and Khan (2010) who studied the pathological responses of progeny of hens kept on ochratoxin A and aflatoxin contaminated feed respectively. Tedesco *et al*. (2004), also reported improved weight gain and feed intake in milk thistle treated group as compared to birds raised on aflatoxin contaminated feed alone. Similarly, Kalorey *et al*. (2005) reported improvement in feed intake by milk thistle in aflatoxicosis, which are in agreement to the present findings.

Post-mortem lesions: Post-mortem lesions included pale, enlarged (swollen), yellow friable livers with pinpoint haemorrhages, swollen kidneys and atrophy of bursa and thymus in broiler suffering from aflatoxicosis. Heart showed hydropericardium, intestines revealed haemorrhagic enteritis. These findings are in conformity with Denli and Okan (2006). Pathological changes in liver and other organs were of milder degree in milk thistle fed birds as compared to birds raised on contaminated feed only and those raised on toxin binder. Similar findings were recorded by Hussain *et al*. (2008), who conducted clinico-pathological studies of experimentally induced aflatoxicosis in broiler chicks.

Table 1: Total serum protein, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in experimental birds

Group	Total serum protein (g/dl)	ALP (µ/l)	ALT (µ/l)	AST (µ/l)
A	5.0±0.1^{a}	465.1±14.7^{b}	48.3±3.4^{b}	36.0±3.6^{b}
B	2.4±0.1^{c}	671.5±15.6^{a}	64.6±1.7^{a}	60.1±2.2^{a}
C	4.7±0.1^{b}	469.2±12.1^{b}	51.0±3.2^{b}	39.5±4.0^{b}
D	5.0±0.1^{a}	459.4±12.5^{b}	46.0±3.6^{b}	36.1±3.2^{b}
Vaccination				
Vac	4.2±0.3	532.7±28.3^{a}	47.0±2.7^{b}	38.1±4.1
Non-Vac	4.3±0.3	499.9±27.9^{b}	57.9±2.3^{a}	47.8±2.7
Inter-action				
A x Vac	4.9±0.1	483.4±6.1	42.3±2.4	30.6±4.9
A x Non-Vac	5.0±0.1	446.8±26.7	54.3±4.0	41.3±3.8
B x Vac	2.5±0.1	693.1±20.5	61.3±1.7	58.6±4.4
B x Non-Vac	2.2±0.1	650.0±18.2	68.0±1.1	61.6±2.0
C x Vac	4.6±0.1	482.0±3.0	45.6±2.7	32.3±4.8
C x Non-Vac	4.8±0.1	456.4±23.6	56.3±4.0	46.6±2.4
D x Vac	4.8±0.1	472.3±7.2	39.0±1.7	30.6±4.7
D x Non-Vac	5.1±0.0	446.5±23.8	53.0±3.7	41.6±1.2

Mean±SE within a column with different superscripts are significantly different at P = 0.05.

Table 2: Feed intake (g), body weight gain (g) and feed conversion ratio (FCR) in Experimental birds

Group	Feed intake	Weight gain	FCR
A	2700.3±40.5^{a}	1129.5±11.7^{a}	2.39±0.03^{b}
B	2286.3±26.9^{c}	804.3 ±8.7^{c}	2.84±0 .03^{a}
C	2571.1±42.0^{a}	1054.6±23.2^{b}	2.44±0 .05^{b}
D	2564.2±44.3^{b}	1078.3±21.3^{b}	2.38±0.02^{b}
Vaccination			
Vac	2547.8±49.7	1020.9±39.2	2.52±0.06
Non-vac	2509.7±56.0	1012.5±38.1	2.51±0.05
Inter-action			
A x Vac	2686.1±64.6	1128.5±20.1	2.38±0.06
A x Non-Vac	2712.5±105.3	1130.5±25.9	2.40±0.03
B x Vac	2310.2±29.6	803.4±6.0	2.87±0.04
B x Non-Vac	2262.4±37.5	805.2±5.8	2.81±0.04
C x Vac	2623.7±32.0	1065.8±24.1	2.46±0.04
C x Non-Vac	2520.5±56.2	1043.5± 21.4	2.41±0.10
D x Vac	2577.0±85.5	1086.0±17.1	2.37±0.05
D x Non-Vac	2549.5±39.3	1070.6±17.0	2.38±0.0

Mean±SE within a column with different superscripts are significantly different at P = 0.05.

Conclusions: The research study concluded that milk thistle at the rate of 10 g/kg feed could be effectively utilized as hepatoprotective and growth promoter in the presence of hepatotoxic aflatoxin B_1 in the feed.

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