



Protective Effects of Milk Thistle (*Silybum marianum*) against Aflatoxin B₁ in Broiler Chicks

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ABSTRACT : Aflatoxin-contaminated feed cause mortality, suppression of the immune system, reduced growth rates and losses in feed efficiency. This research study was planned to investigate the immunomodulatory and growth promoting effect of milk thistle as feed additive against aflatoxin B₁ in broiler chicks at NWFP Agricultural University Peshawar, Pakistan. Two hundred and forty (240) day old broilers chicks were randomly assigned into four major groups AfF, aflatoxin free feed; Aflatoxin B₁ was present in the feed at the levels of 80-520 µg/kg of the feed in the remaining three groups. Aflatoxin contaminated feed was provided for 5 weeks. Group AfB was supplemented with toxin binder “Mycoad” at 3 g/kg of feed and group AfT was supplemented with milk thistle at 10 g/kg of feed. Each group was further sub divided into two sub-groups, vaccinated against ND (Newcastle disease), IB (Infectious bronchitis) and IBD (Infectious bursal diseases) according to recommended schedule of vaccination or non vaccinated. Each sub group carried three replicates with 10 chicks per replicate. Chicks were reared in pens in an open sided house. Supplementary heat was provided to all the chicks during brooding period. Mean body weight gain and dressing percentage were significantly ($p < 0.05$) higher in group AfF, followed by AfT, AfB and Af. Weight gain and dressing percentage was the same in group AfB and AfT, while it was significantly lower in group Af. Feed intake, breast, thigh and leg weight were found significantly ($p < 0.05$) higher in group AfF, followed by AfB, AfT and Af. Significantly lower (better) FCR value was recorded in group AfT. Water intake was significantly ($p < 0.05$) higher in group AfT and AfF as compared to other groups. Mortality was significantly ($p < 0.05$) higher in group Af. Mean bursa and thymus weights were found significantly ($p < 0.05$) higher in group AfF, AfB and AfT followed by Af, while higher spleen weight was recorded in group AfT. Mean antibody titer against ND, IB and IBD was significantly ($p < 0.05$) higher in group AfT, as compared to other groups. It is concluded that milk thistle at 10 g/kg of feed could effectively be utilized as immunostimulant and growth promotant in the presence of immunosuppressant aflatoxin B₁ in the feed. (**Key Words** : Immunostimulant, Growth Promotant, Milk Thistle, Toxin Binder, Broiler, Aflatoxin, Poultry)

INTRODUCTION

Chicken meat is an important source of nutrients for human consumption. Broiler feed is exposed to various contaminants during the process of production, transportation and storage. Moisture is the most important factor favoring the growth of fungus during these stages while the hygienic condition and quality control measure are normally poor in the area under study. One of the important injurious agents is mycotoxins as it has hepatotoxic, hepatocarcinogenic, mutagenic and teratogenic effects in many animal species (IARC, 1987). Mycotoxins are metabolites from moulds. Aflatoxins, a group of closely

related biologically active mycotoxins, are produced by strains of *Aspergillus flavus* and *Aspergillus parasiticus*. Even though, 18 different Aflatoxins have been identified, only Aflatoxins B₁, B₂, G₁ and G₂ have been detected as natural contaminants of feedstuffs. In a previous study Tedesco et al. (2004) the peanuts and cereals have been reported as having afore-mentioned natural contaminants.

Aflatoxins have been shown to cause Immunosuppression (Rahim et al., 1999; Kalorey et al., 2005) and growth retardation (Arshad et al., 1992). Pathological toxic lesions in liver include pale, enlarged, yellow friable livers with pinpoint haemorrhage (Rahim et al., 1999), hepatomegaly, pale discoloration of liver, mottling of liver, severe fatty change, necrosis, leucocytic infiltration in portal triads, petechial haemorrhages (Arshad et al., 1992), fibrosis, bile duct hyperplasia (Asim et al., 1990) and high mortality (Rahim et al., 1999).

Milk thistle is a locally available herb throughout

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Received November 17, 2010; Accepted February 18, 2011

Pakistan and has great medicinal properties. Milk thistle has hepatoprotective, anti-inflammatory, cytoprotective and anti-carcinogenic effects (Manna et al., 1999). Milk thistle protects birds against adverse effects of aflatoxin B₁ (Grizzle et al., 1999; Tedesco et al., 2004; Kalorey et al., 2005). Milk thistle also serves as immunostimulant (Grizzle et al., 1999; Thyagarajan et al., 2002; Wilasrusmee et al., 2002). Milk Thistle has been reported to increase lymphocyte proliferation which is associated with an increase in interferon gamma, interleukin (IL)-4 and IL-10 cytokines (Wilasrusmee et al., 2002). Silymarin stabilize hepatocyte and other cell membranes and stimulate macromolecular and protein synthesis. Additionally it inhibits neutrophil-mediated histamine release, lipoxygenase and prostaglandin synthetase, and leukotriene synthesis.

Keeping in view the medicinal value of Milk thistle, this research study was conducted to investigate the effect of Milk thistle on the growth and immune performance of broiler chicks against Newcastle disease (ND), infectious bursal disease (IBD) and infectious bronchitis (IB).

MATERIALS AND METHODS

The research study was conducted at NWFP Agricultural University, Peshawar poultry farm to investigate the immunomodulatory and growth promoting effect of milk thistle against aflatoxin B₁ in broiler chicks. Moreover, detection of aflatoxin B₁ and their level in feed was carried out in the Nutrition Laboratory of Directorate of Livestock Research and Development, Peshawar.

Experimental design

The experiment was conducted in completely randomized block design (CRBD) with two factors i.e. i) level of milk thistle and ii) level of vaccine.

Two hundred and forty (240) starbrow, commercial day-old broiler chicks of approximately the same weight were purchased from the local market. Chicks were divided into four groups AfF, Af, AfB and AfT and each group was further divided into two sub-groups for different vaccination. Each sub-group carried three replicates with 10 chicks per replicate. The birds were raised in 4×4 square foot wooden pens on conventional deep litter, open sided housing system. All the pens were located in the same house in order to have identical environment, where each pen was provided with separate feeder and drinker. Chicks were brooded in the same pens and artificial supplementary heat was provided during the brooding period to meet the requirements of the chicks. Feed and water was offered on daily basis and the refusal was measured next morning. Birds were examined for health status on daily basis. Temperature was recorded and maintained on daily basis. Group AfF was kept as control while group Af was fed

contaminated feed, group AfB was fed contaminated feed along with Toxin binder "Mycoad" at the dose rate of 3 g/kg feed and group AfT was provided contaminated feed along with milk thistle at the dose rate of 10 g/kg feed.

Production of aflatoxin B₁ contaminated poultry feed

The aflatoxigenic strain of *Aspergillus flavus* isolated previously on Sabouraud agar from field cases of aflatoxicosis as well as from contaminated feed samples was used for the production of aflatoxins during the course of the experiment. The field cases comprised aflatoxicosis infected broilers received at Veterinary Research Institute, Peshawar, for diagnosis purposes, while the feed samples were provided by the owners of the infected birds and/or collected from the feed suppliers in the relevant market. Broth culture of the aflatoxigenic stain of *Aspergillus flavus* was evenly inoculated aseptically on freshly prepared Sabouraud agar plates with the help of sterile cotton swabs. The plates were incubated in incubator at 28°C for 7 days. A briefly thick growth of spores was produced during this period. The 50 kg poultry feed bag was inoculated with the above culture and was thoroughly mixed. The feed was moistened with water and again mixed thoroughly. The feed bags were kept in store at minimum of 24°C and moisture level above 17.5% to enhance the fungal growth. Contaminated feed was prepared for each week separately. Keeping the amount of both the inoculum and feed constant and a representative feed sample for every week was subjected to analysis for aflatoxins (Rahim et al., 1999). Analysis of experimentally contaminated feed by TLC method showed that aflatoxin B₁ was present in the feed at the levels of 80-520 µg/kg of the feed. The TLC plate was coated with slurry of silica gel to a thickness of 750 µm, dried in air and activated at 100°C for two hours in oven. Chloroform extract of feed, 200 µl, was spotted on the plate in the form of 10 µl spots spreading over an area of 6 cm in width at a height of 2 cm from the baseline. The spot was air dried. The plate was developed using diethylether as developer to a liquid front of 12 cm from the baseline in an equilibrated tank. The TLC plate was removed from the tank, air dried and examined under UV light. The plate was developed in an equilibrated tank using chloroform method developer (50+1 ratio) to a liquid front of 12 cm from the baseline. It was removed from the tank, dried and redeveloped to the same liquid front in the same developer. The plate was viewed under UV light and the position of blue and green fluorescing spot of B1, B2 and G1, G2 were marked. The spots were scrapped and silica gel of each component was collected in a separate glass dish. The gel was extracted with cold methanol method for 3 minutes and the extract filtered into a small beaker. The gel was thrice washed with methanol, making the combined methanol filtrates up to 5 ml. The methanolic extract was examined in

a Double Beam spectrophotometer using methanol as blank. The densities of aflatoxins were measured at 363 and 420 Angstrom. The corrected optical density was calculated by subtracting the value at 420 Angstrom from 363 Angstrom. The quantity of aflatoxin was then calculated.

Preparation of milk thistle powder

The seeds of milk thistle were collected from Warsak and adjoining tribal areas of Michini, Mohmand Agency of Northern Pakistan in summer season. After drying, it was ground to powder form with the help of electric grinder. Powdered milk thistle was mixed in feed at the rate of 10 g/kg feed. Toxin binder (Mycoad) was mixed in feed at the rate of 03 g/kg feed.

Data was recorded for body weight gain (weekly basis), feed intake (daily basis), dressing percentage (at the end of experiment), water consumption (daily basis) and mortality. Feed intake was calculated by subtracting feed refusal from feed offered while water intake was calculated by subtracting water refused from water offered on daily basis. For determination of dressing percentage, one bird from each replicate was live weighed and slaughtered, head, feet, and all internal visceral organs including abdominal fat were removed and then the dressed body was weighed. The dressed body weight was then expressed in terms of dressing percentage by using the following formula:

$$\text{Dressing percentage} = (\text{Live weight}/\text{Dressed weight}) \times 100$$

Feed conversion ratios (FCR) were calculated at the end

of experiment. ND, HI antibodies were estimated using the haemagglutination inhibition tests described by Alexander and Chettle (1997), whereas antibody response against IB and IBD was estimated using ELISA (Marquardt et al., 1980). Tissue samples (bursa of fabricius, thymus and spleen) from chicks in each sub-group were collected on last day of experiment.

Statistical analysis

The data was statistically analyzed with the standard procedures of analysis of variance (ANOVA), using completely randomized block design as suggested by Steel and Torrie (1981). The statistical packages SPSS-10 (1999) were used to perform the above analysis on computer.

RESULTS AND DISCUSSION

The results obtained are presented under various sections as follows:

Body weight gain

Mean body weight gain per chick for the four experimental groups and for vaccinated and non vaccinated sub-groups are presented in Table 1. The data when subjected to statistical analysis, showed significant ($p < 0.05$) difference among the groups and non significant between vaccinated and non vaccinated chicks. Interaction effects were also non-significant. Maximum body weight gain was observed in group AfF, receiving normal feed, followed by group AfT (80-520 $\mu\text{g}/\text{kg}$), receiving aflatoxin B₁ contaminated feed with milk thistle. The body weight gain

Table 1. Mean water intake (ml), feed intake (g), body weight gain (g) and FCR in broiler chicks fed normal feed, aflatoxin contaminated feed, toxin binder "Mycoad" and milk thistle*

Group	Water intake	Feed intake	Weight gain	FCR	Mortality
AfF	7,705.14 ^a ±259.35	2,700.36 ^a ±55.57	1,129.53 ^a ±14.70	2.39 ^b ±0.03	0 ^c
Af	7,205.71 ^b ±233.87	2,287.36 ^c ±23.91	804.36 ^c ±3.78	2.84 ^a ±0.03	2.83 ^a ±0.30
AfB	7,548.90 ^{ab} ±109.66	2,572.14 ^a ±37.02	1,054.67 ^b ±15.29	2.44 ^b ±0.05	1.33 ^b ±0.61
AfT	7,911.83 ^a ±97.00	2,563.28 ^b ±42.55	1,078.33 ^b ±11.33	2.37 ^b ±0.02	0.83 ^{bc} ±0.30
Vaccination					
Vac	7,725.22±133.81	2,549.82±49.72	1,020.95±39.24	2.52±0.06	1.00±0.34
Non-vac	7,460.57±153.62	2,511.75±56.06	1,012.50±38.17	2.50±0.05	1.50±0.43
Interaction					
AfF×Vac	8,149.71±343.18	2,687.17±64.62	1,128.54±20.13	2.38±0.06	0
AfF×Non-Vac	7,260.57±144.58	2,713.55±105.33	1,130.52±25.96	2.40±0.03	0
Af×Vac	7,478.04±373.58	2,311.27±29.63	803.44±6.00	2.87±0.04	2.66±0.33
Af×Non-Vac	6,933.37±244.43	2,263.45±37.55	805.28±5.87	2.81±0.04	3.00±0.57
AfB×Vac	7,562.80±32.31	2,623.77±32.07	1,065.80±24.16	2.46±0.04	0.66±0.66
AfB×Non-Vac	7,535.00±242.68	2,520.52±56.21	1,043.53±21.47	2.41±0.10	2.00±1.00
AfT×Vac	7,710.33±40.08	2,577.07±85.53	1,086.00±17.15	2.37±0.05	0.66±0.33
AfT×Non-Vac	8,113.33±69.60	2,549.50±39.36	1,070.67±17.02	2.383±0.00	1.00±0.57

* Means within the same column with different superscripts are significantly different at $\alpha = 0.05$.

was severely depressed in birds receiving aflatoxin contaminated feed without milk thistle and toxin binder (Group-B). Non significant differences were observed between group AfB and AfT. It is evident from the above observations that body weight gain was the lowest in aflatoxin B₁ treated birds. In the Aflatoxin B₁ and milk thistle treated birds, body weight gain was higher with respect to birds receiving AFB₁ alone and not different from the toxin binder treated birds. The results of the present study are in agreement with the findings of Tedesco et al. (2004), who reported that addition of silymarin phytosome in the feed at 600 mg/kg of body weight resulted in an increase of 14.83% in body weight. This increase was lower than the present value of 31.12%. Similarly, Gowda and Sastry (1998) also confirmed significant ($p < 0.05$) improvement of milk thistle on body weight gain and attributed the effect to antioxidant activity that stimulated protein synthesis by bird's enzymatic system. Higher weight gain was also reported by Chakarverty and Parsad (1991), in milk thistle supplemented group. The exact mechanism of improving body weight is not well established; however this effect might be due to the improved immune function of the birds (Table 3) receiving milk thistle. Relative weight of the lymphoid organs and antibody titers against ND, IB and IBD were improved in milk thistle fed group, which are indicative of improved function of the immune system. Avian thymus and bursa of fabricius play an important role in the development of immunological competence as neonatal surgical or embryonic hormonal bursectomy in chicken greatly reduced subsequent antibody forming capacity (Mueller et al., 1960).

Feed and water intake

Mean feed and water intake per chick are presented in Table 1. Significant ($p < 0.05$) differences were recorded among the treated groups and non significant between vaccinated and non-vaccinated groups. Interaction effects were also non-significant. Water intake was significantly higher in group AfF and AfT than other groups. Feed intake was significantly higher in control group AfF and was similar to group AfB. Feed intake was lowest in group Af. Presence of aflatoxin B₁ in feed depressed feed intake (group Af). On the other hand, presence of milk thistle in aflatoxin contaminated feed resulted in improved feed intake, which is at par with control. The higher feed intake in control group did not caused proportionate increase in body weight gain therefore, resulted in lower efficiency of feed utilization. The results of this study support the findings of Kalorey et al. (2005), who reported that milk thistle improved feed intake in presence of aflatoxin B₁ in feed. Our results are correlated with Tedesco et al. (2004), who observed improved feed intake in milk thistle treated group as compared to birds raised on aflatoxin

contaminated feed only.

In the present study feed intake was improved by 12.45% in milk thistle fed group (group AfT) as compared 12.06% in the toxin binder group and 18.06% in the toxin free group. This shows sufficient improvement in performance of broilers with the supplementation of milk thistle. Similarly, Tedesco et al. (2004) reported 22.29% improvement in feed intake, having silymarin phytosome at 600 mg/kg of body weight. The greater improvement in feed intake may be due to the pure form of silymarine phytosome while in the present study milk thistle seeds as whole were used which may contain anti-nutritional factors leading to depressed appetite.

Feed conversion ratio

Average feed conversion ratio (FCR) was 2.39, 2.84, 2.44 and 2.37 for the four experimental treated groups AfF, Af, AfB and AfT, while it was 2.52 and 2.50 for vaccinated and non vaccinated groups, respectively (Table 1). FCR was significantly ($p < 0.05$) affected by the treatments but non significant differences were observed between vaccinated and non vaccinated chicks. Interaction effects were also non-significant. FCR values were significantly higher (poor) in group Af and were the same in all other groups. Numerically feed efficiency was the best in milk thistle fed group. The results of our study are in agreement with the findings of Tedesco et al. (2004), who observed better feed efficiency response (2.527) in aflatoxin B₁ contaminated feed with milk thistle with respect to birds receiving aflatoxin B₁ contaminated feed alone (2.929), during the last two weeks. Similar results were also reported by Chakarverty et al. (1991) and Zahid and Durrani (2007), who fed milk thistle to broilers and observed efficient feed conversion ratio in broilers. Zahid and Durrani (2007) fed milk thistle to broilers at the rate of 15 g/kg feed and found better FCR (2.2) as compared to the control group (2.4).

Dressing percentage

Mean dressing percentage was significantly affected by treatments, while vaccination and interaction effects were non significant (Table 2). Mean dressing percentage was lower ($p < 0.05$) in birds raised on aflatoxin contaminated feed alone (group Af), while no significant ($p < 0.05$) difference was observed in group AfF, AfB and AfT. Zahid and Durrani (2007) reported similar findings, who fed different levels of milk thistle to broilers and found significantly higher dressing percentage at the level of 15 g/kg feed. In the present study an increase of 5.96% in dressing percentage was recorded while Zahid and Durrani (2007) reported 3.92% improvement in dressing percentage.

Weight of different body organs

Differences in breast, thigh and leg muscles were

Table 2. Mean dressing percentage, breast (g), thigh (g), leg weights (g) in broiler chicks fed normal feed, aflatoxin contaminated feed, toxin binder "Mycoad" and milk thistle*

Group	Dressing %	Breast weight	Thigh weight	Leg weight**
AfF	58.11 ^a ±0.92	275.60 ^a ±4.01	72.91 ^a ±1.20	57.21 ^a ±1.17
Af	54.50 ^b ±0.25	213.00 ^b ±5.15	60.48 ^c ±0.98	44.10 ^b ±0.67
AfB	57.64 ^a ±0.55	266.91 ^a ±4.20	70.71 ^{ab} ±0.37	53.58 ^a ±2.67
AfT	57.74 ^a ±0.68	262.60 ^a ±5.35	69.95 ^b ±0.42	53.11 ^a ±1.35
Vaccination				
Vac	57.47±0.66	253.13±7.96	69.18±1.51	52.51±2.08
Non-vac	56.53±0.53	255.92±8.06	67.85±1.54	51.49±1.52
Interaction				
AfF×Vac	58.73±0.72	273.70±4.05	73.50±2.43	57.73±0.71
AfF×Non-Vac	57.50±1.83	277.50±7.77	72.33±0.98	56.70±2.47
Af×Vac	54.01±0.16	211.66±7.88	61.60±1.70	43.46±1.17
Af×Non-Vac	54.99±0.24	214.33±8.29	59.36±0.80	44.73±0.70
AfB×Vac	58.25±0.64	261.50±3.50	71.23±0.57	55.76±5.29
AfB×Non-Vac	57.04±0.86	272.33±6.85	70.20±0.32	51.40±1.73
AfT×Vac	58.89±0.82	265.66±10.88	70.40±0.60	53.10±2.31
AfT×Non-Vac	56.59±0.59	259.53±3.95	69.50±0.55	53.13±1.95

* Means within the same column with different superscripts are significantly different at $\alpha = 0.05$. ** One leg weight.

significant among the groups and non-significant between vaccinated and non vaccinated chicks. Interactive effects were also non-significant (Table 2). Zahid and Durrani (2007) reported similar findings, who fed different levels of milk thistle to broilers and found significantly higher breast and thigh weights at the level of 15 g/kg feed. They reported higher (33.65 and 64.89%) improvement in breast and thigh muscles, respectively which are higher than our

values. The greater improvement in breast and thigh muscles may be due to the high level of milk thistle while in the present study milk thistle seeds were used at the level of 10 g/kg feed.

Weight of lymphoid organs

Bursa weight : Bursa weight data showed significant ($p < 0.05$) differences among treatments and non significant

Table 3. Mean weight of lymphoid organs (g) and antibody titer against ND, IB and IBD in broiler chicks fed normal feed, aflatoxin contaminated feed, toxin binder "Mycoad" and milk thistle*

Group	Bursa	Spleen	Thymus	ND	IB	IBD
AfF	1.85 ^a ±0.06	1.38 ^{ab} ±0.07	3.20 ^a ±0.12	2.0 ^c ±0.63	4.56 ^c ±0.87	639.0 ^c ±142.3
Af	1.15 ^b ±0.06	0.88 ^c ±0.04	1.93 ^b ±0.11	3.0 ^d ±0.22	0.66 ^d ±0.33	209.0 ^d ±77.6
AfB	1.85 ^a ±0.05	1.28 ^b ±0.09	3.28 ^a ±0.19	3.3 ^b ±0.61	4.83 ^b ±0.30	1,066.50 ^b ±30.7
AfT	1.78 ^a ±0.04	1.56 ^a ±0.08	3.16 ^a ±0.17	4.5 ^a ±0.22	6.16 ^a ±0.30	1,524.67 ^a ±177.5
Vaccination						
Vac	1.67±0.07	1.30±0.08	2.84±0.17	3.42 ^a ±0.46	4.16 ^a ±0.64	1,061.67 ^a ±176.7
Non-vac	1.64±0.11	1.25±0.09	2.95±0.21	1.75 ^b ±0.50	2.75 ^b ±0.76	657.91 ^b ±135.0
Interaction						
AfF×Vac	1.76±0.12	1.23±0.08	3.13±0.12	3.33±0.33	4.00±0.57	937.66±40.5
AfF×Non-Vac	1.93±0.03	1.53±0.03	3.26±0.24	0.66±0.33	0.33±0.33	340.33±102.4
Af×Vac	1.26±0.03	0.96±0.03	1.96±0.08	1.00±0	1.00±0.57	298.0±142.2
Af×Non-Vac	1.03±0.06	0.80±0.05	1.9±0.23	0	0.33±0.33	120.0±45.1
AfB×Vac	1.86±0.08	1.36±0.16	2.93±0.27	4.66±0.33	5.33±0.33	1,111.67±34.4
AfB×Non-Vac	1.83±0.06	1.20±0.11	3.63±0.03	2.00±0	4.33±0.33	1,021.33±38.6
AfT×Vac	1.80±0.05	1.66±0.08	3.33±0.23	4.66±0.33	6.33±0.33	1,899.33±111.4
AfT×Non-Vac	1.76±0.06	1.46±0.14	3.0±0.26	4.33±0.33	6.00±0.66	1,150.00±69.3

* Means within the same column with different superscripts are significantly different at $\alpha = 0.05$.

between vaccinated and non vaccinated groups (Table 3). Interaction effects were also non-significant. Bursa weight was significantly lower in group Af, while non significant differences were observed in groups AfF, AfB and AfT. Aflatoxin contaminated feed significantly reduced bursa weight (group Af), causing interference with antibody production. However, milk thistle supplemented feed restored the normal weight of bursa of fabricius (group AfT). The findings of the present study are supported by Tedesco et al. (2004), who reported reduced bursa weight in broilers by feeding aflatoxin contaminated feed, while increased weight and morphology of bursa in milk thistle feeded groups. Kalorey et al. (2005) also reported the protective role of milk thistle against aflatoxicosis on weight of Bursa of fabricius.

Milk thistle has been reported to support the immune system through its powerful antioxidant, free-radical scavenging action, its ability to preserve the supply of another important antioxidant, glutathione, as well as direct effects on immune cells (Basaga et al., 1997). Silymarin, as antioxidant, has protective action against the oxidative damages on the immune organs (Bursa, spleen and thymus) and immune cells which cause immunosuppression.

Spleen weight : Spleen weight was significantly higher in group AfT, followed by AfB, AfF and Af, respectively (Table 3). As evident from the observations, aflatoxins significantly ($p < 0.05$) reduced spleen weight and milk thistle was more efficient to protect spleen against adverse effect of aflatoxin as compared to synthetic toxin binder. Similar findings have been reported by Kalorey et al. (2005) and Tedesco et al. (2004) by feeding aflatoxin contaminated

feed and milk thistle to broilers.

Thymus weight : Mean thymus weight was significantly affected by treatments and non significantly by vaccination. Interactive effects were also non-significant ($p > 0.05$) (Table 3). Lowest thymus weight was observed in birds raised on aflatoxin contaminated feed alone (group Af), while non significant differences were recorded in groups AfF, AfB and AfT (Table 3). Findings of the present study are supported by Tedesco et al. (2004), who reported reduced weight of thymus by feeding Aflatoxin B₁ contaminated feed to broilers and protective role of milk thistle on thymus weight in aflatoxicosis. Similar findings were also reported by Kalorey et al. (2005), who reported that feed added milk thistle preserved morphology and weight of lymphoid organs (thymus, bursa and spleen) in aflatoxicosis.

Antibody titer

Mean antibody titer of broilers against ND, IB and IBD is given in Table 3. Serum antibody titer for ND, IB and IBD was significantly higher in group AfT (milk thistle treated group), while lowest titers were recorded in group Af. It is clear from the above observations that aflatoxin caused severe immuno-suppression due to reduction in phagocytic activity of blood monocytes, depressed complement activity, hence depressed opsonization and phagocytic activity. However, treatment with milk thistle protected the reduction of humoral immune response in broiler due to aflatoxin B₁ in feed. Milk thistle treated birds also revealed better antibody titer than toxin binder treated group. The results of the present study are supported by Grizzle et al. (1999), Tedesco et al. (2004) and Kalorey et al.

Table 4. ANOVA parameters for changes in performance parameters, lymphoid organs and antibody titers in broiler chicks fed various diets*

Source	DF	F value	p value	F value	p value	F value	p value
		----- Weight gain -----		----- Feed intake -----		----- FCR -----	
Group	3	120.99	0.0001	15.81	0.0001	32.06	0.0001
Vac.	1	0.41	0.5308	0.76	0.3971	0.26	0.6182
Group X Vac.	3	0.22	0.8830	0.37	0.7723	0.27	0.8450
		----- Bursa -----		----- Spleen -----		----- Thymus -----	
Group	3	45.08	0.0001	16.01	0.0001	19.88	0.0001
Vac.	1	0.43	0.5201	0.65	0.4308	0.56	0.4638
Group X Vac.	3	2.59	0.0885	2.75	0.0768	2.31	0.1157
		----- ND -----		----- IBD -----		----- IB -----	
Group	3	85.60	0.0001	94.04	0.0001	64.07	0.0001
Vac.	1	80.00	0.0001	48.05	0.0001	20.64	0.0003
Group X Vac.	3	10.13	0.0006	7.52	0.0023	5.98	0.0062
Error	16						
Total	23						

* Group AfF was kept as control while group; Af was fed contaminated feed; group AfB was fed contaminated feed along with Toxin binder "Mycoad" at the dose rate of 3 g/kg feed; group AfT was provided contaminated feed along with milk thistle at the dose rate of 10 g/kg feed.

(2005), who reported the immunomodulatory role of milk thistle in the presence of immunosuppressant aflatoxin in feed. Similarly Wilasrusmee et al. (2002) and Thyagarajan et al. (2002) also reported the immunostimulatory effect of milk thistle. Basaga et al. (1997) reported that milk thistle supports the immune system through its powerful antioxidant, free-radical scavenging action, its ability to preserve the supply of another important antioxidant, glutathione, as well as direct effects on immune cells. Silymarin protect the immune cells and organs (Bursa, spleen and thymus) against the oxidative damages which cause immunosuppression.

Mortality

Mean mortality of broilers is presented in Table 1. The data when subjected to statistical analysis, showed significant ($p < 0.05$) difference among the groups and non significant between vaccinated and non vaccinated chicks. Interaction effects were also non-significant. Significantly higher mortality was observed in group Af, followed by AfB, AfT and AfF, respectively. The results of the present study are supported by the findings of Siddique and Javed (1989), who reported 10.6 percent mortality in broiles, raised on feed with 30-50 ppb of aflatoxins. Findings of the present research trial are also in line with those of Sabri et al. (1989), who reported 12.47 percent mortality in broiles, raised on feed with 22-96 ppb of aflatoxins.

Post-mortem lesions

Post-mortem lesions included pale, enlarged (swollen), yellow friable livers with pinpointed haemorrhages, swollen kidneys and atrophy of bursa and thymus in broiler suffering form aflatoxicosis. Heart showed hydro pericardium, intestines revealed haemorrhagic enteritis. These findings are in conformity with Rahim et al. (1999). 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 Arshad et al. (1992); they conducted clinico-pathological studies of experimentally induced aflatoxicosis in broiler chicks. Similar results were also observed by Sabri et al. (1989); who studied prevalence and pathology of mycontoxicosis in broiler chicks in and around Faisalabad.

It is concluded that milk thistle at 10 g/kg of feed may effectively stimulate the immune function and growth performance in the presence of immunosuppressant aflatoxin B₁ in the feed.

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