Toxicopathological effects of moldy feed in commercial white leghorn layers and its amelioration with milk thistle seed

Aminullah[1], Arsala Khan[1], Muhammad Kashif Khan[2], Muhammad Zeeshan[3], Hashmat Ullah[4], Farhan Anwar Khan[5], Zubair Luqman[6], Usman Ghani[7]

[1] *Department of Livestock and dairy development (Extension wing) Khyber Pakhtunkhwa.*

[2] *Department of Pathology, Faculty of Veterinary Science, The University of Agriculture Peshawar, Pakistan.*

[3] *Department of Anatomy & Histology, University of Veterinary and Animal Sciences, Lahore, Pakistan.*

[4] *Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore, Pakistan.*

[5] *Department of Animal Health, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan.*

[6] *University College of Veterinary & Animal Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan.*

[7] *Livestock Production Officer District lower Dir.*

Abstract

The current experiment was conducted to study the toxic pathological effects of moldy feed and it's amelioration by concurrent dietary incorporation of MTS against mycotoxins in white leghorn hens (WLH). For this purpose, a total of one hundred, 40 weeks old WLH were randomly divided into five groups i.e. A, B, C, D and E with each group containing 20 hens. Group B received moldy feed containing ochratoxin A 56 microgram/kg and aflotoxin B1 136 microgramg/kg, groups C to E were kept on moldy feed with different concentrations of MTS i.e., 0.5%, 1% and 2.0% respectively, while group A served as a negative control. The birds in all groups were observed for manifestation of clinical signs, physical parameters like body weight gain, eggs weight and egg production were noted. All the birds at end of the experiment were subjected to necropsy for evaluation of gross and histological lesions in various organs, while eight randomly selected birds were used for hematology. The birds in group A were active revealed no clinical signs. All the birds in group B were anorexic with significant decrease in body weight gain, egg production and egg's weight, while the relative weight of liver, kidneys, heart and spleen was significantly increased in group B and C. TEC, TLC, Hb and HCT were significantly decreased in group B and C as compared to all other groups. The gross lesions observed in group B and C include enlarged, friable, pale light color liver, enlarged kidneys, presence of ecchymotic hemorrhages on surface of liver, kidneys, heart and spleen, while in group D and E the severity of these lesions were decreased. Similarly the histological lesions observed in group D and E were also greatly reduced as compared to group B and C. It is concluded from the current study that addition of milk thistle seed in moldy feed with the concentration of 1 to 2 % has almost completely ameliorated the toxic effect of mycotoxicosis in poultry.

Index Terms— Toxicopathological effects, moldy feed, white leghorn layers, amelioration, milk thistle seed (MTS)

***Corresponding author:** drzubairvet@hotmail.com / zubair.luqman@iub.edu.pk

INTRODUCTION

Poultry is one of the major and exciting segments of agriculture industries in Pakistan contributed 1.3 percent in GDP with a healthy annual growth rate @ 8-10 percent that reflects its innate potential (Pak economy survey 2014). Poultry feeds having agricultural products as their basal ingredients, are greatly contaminated by fungi (Saleemullah et al., 2006) therefore, to uphold and sustain the growth of this industry in the country, it is important to provide best quality cereals for preparation of Poultry feed. Poultry feed contamination by Aflotoxins is one of the key issues in subtropical regions round the glob including Pakistan (Khan et al., 2013). Hot and humid climatic conditions in our country are supportive for spread of fungi (Nazir et al., 2014). Aspergillus flavus and Aspergillus parasiticus are the predominant fungal species responsible for aflatoxins production in feed (Yu et al., 2004). These toxins are further divided into four subgroups i.e., B1, B2, G1 and G2. Among these Aflatoxin B1 is the most toxic one, beside with this Aspergillus species can also produce ochratoxins that exist in three secondary forms such as OTA, OTB, and OTC (Bayman et al., 2002). The mycotoxins results in deterioration of consumer's health due to passage of toxin's residues in poultry products (Ishfaq et al., 2014). Moldy feed usually results in depressed immunity and impaired liver function in Poultry and human as well (Hussain et al., 2008; Hassan, 2010; Khan et al., 2010), however; the severity of these effects depends upon level of toxins and duration of exposure to moldy feed (Hussain et al., 2008).

There are numerous approaches to avoid the contamination of food by mycotoxins (Kabak et al., 2006). Milk thistle (MTS) a medicinal herb is found in different areas of the world which has been traditionally used for the treatment of hepatitis and liver cirrhosis as a natural medicine (Giese, 2001). Recent reports have been shown that silymarin, an extract of MTS has remarkably high anti-tumor activity in protecting of hepatic tissues (Lee et al., 2006). Silymarin comprises of a composite of six flavonolignans silychristin, silydianin, silybin A, silybin B, isosilybin A and isosilybin B (Radjabian, T. et al. 2008). Among these silybin is the major constituent of silymarin showing most of the biological actions (Saller et al., 2007; Muhammad et al., 2012; Ahmad et al., 2012). Silymarin has also been successfully used for hepatitis treatment (Mayer and Myers, 2005). It regulates the intracellular glutathione level, stabilize the cell membrane thus preventing the entry of hepatotoxic agents to hepatocytes, act as a stimulant for liver regeneration and also an inhibitor of transformation of stellate hepatocytes into myofibroblast (Fraschini et al., 2002). Silimayrin has also cyto protective, anti-inflammatory and anti- carcinogenic effects (Shaker et al., 2010; Kiruthiga et al., 2010). Keeping in view the therapeutic value of milk thistle seeds, the current project was lunched to study its ameliorative effects in laying hens against dietary exposure to moldy feed.

MATERIALS AND METHODS

2.1 PREPARATION OF MOLDY FEED AND QUANTIFICATION OF MYCOTOXINS

Moldy feed was prepared by method as described by Stoev et al. (2004). The quantification of OTA and AFB1 was performed by ELISA technique using commercially available kits such as Max Signal Ochratoxin A ELISA test Kit 1036 – 02; Max Signal Aflatoxin B1 ELISA test Kit 1055 – 04, M/S Bio Scientific Corporation, Austin, TX. Level of OTA and AFB1 in moldy feed used in the current experiment was 56 and 136 mg/kg, respectively.

2.2 EXPERIMENTAL DESIGN

A total of one hundred, 40 weeks old white Leghorn hens (WLH) were purchased from commercial farm and kept under standard management conditions. After two days of acclimatization, the birds were divided into five equal groups i.e. A, B, C, D and E with each group containing 20 hens. Experimental feed containing different concentrations of OTA and AFB1 was offered to all of the birds, except group A that served as a negative control. Group B received moldy feed (OTA+AFB1: 56 and 136 mg/kg), while all the groups from C to E were kept on moldy feed along with 5 (0.5%), 10 (1.0%) and 20 (2.0%) g/kg of MTS, respectively.

2.3 CALCULATION OF PHYSICAL PARAMETERS

Birds in all groups were monitored daily for manifestation and severity of clinical signs throughout the experimental trials. An arbitrary score from 0 to 4 were allotted for alertness, feces consistency and feather conditions. The body weight gain, egg production and egg's weight were also, recorded weekly. Data regarding feed intake were taken daily and presented on weekly basis at end of the experiment. All the birds were kept for six weeks.

2.4 HEMATOLOGICAL STUDIES

Randomly selected eight birds from each group were slaughtered at end of the experiment. The blood samples used for hematological studies were collected in EDTA containing vaccutainors. Erythrocyte counts were determined by hemocytometer. Hematocrit was determined by using microhematocrit and hemoglobin concentration was measured by spectrophotometry method as described by Matrai et al. (1987). RBC indices, i.e., MCV, MCH and MCHC were also, determined through method described earlier by Travis et al. (2006).

2.5 GROSS AND HISTOPATHOLOGY

All the birds slaughtered at end of the experiment were subjected to necropsy examination. Gross lesions observed in various organs were noted. Visceral organs including liver, heart, kidney and spleen were weighed and their relative weights were measured as a function of body weight. Tissue samples from liver, kidneys, heart and spleen collected for histopathology were processed through procedure adopted by Abdel Moneim et al. (2009)

2.6 STATISTICAL ANALYSIS

The data obtained were subjected to statistical analysis by ANOVA test and different group means were compared by Duncan's multiple range tests using M-STATC statistical software package (Michigan State University, East Lansing, MI). The level of significance considered was $P < 0.05$.

RESULTS

3.1 PHYSICAL PARAMETERS

At 1st week group C, D and E had significantly higher feed intake, while group B was non- significantly different than group A. At 2nd week all the groups showed non- significant difference from control group. At 3rd week group B was significantly lower than group A, while group C, D and E were also different from control, likely at 4th , 5th and 6th weeks the feed intake in group B was significantly lower while,

groups C, D and E were non- significantly different from that of control group (Table-1). The body weight gain at first, 3rd and 4th weeks of experimental trails in group B, D and E were significantly lower, while that of group C and E was non significantly different than group A. similarly at 2nd week in group B the body weight gain was significantly lower, while that of group C, D and E was non significantly different as compared to group A. Similarly at the end of 5th week the body weight in group B, C, D and E were significantly lower than group A. while, at 6th week group B and C had significantly lower body weight gain than group D and E as compared to control group (Table.2).The percent eggs production at 1st and 2nd week in group D and C was significantly higher than group A, while all other groups were non-significant to each other. Groups B, C and E were significantly lower from control group A. however, the percent egg produced by group B, C at 4th and group B and E at 6th week were significantly lower than group A. similarly at 5th week it was also in significantly lower in group B, D and E. At 1st and 2nd week the egg weight in groups B, C, D and E was non-significantly different than group A while, at 3rd week there was significant decrease in egg weight of groups B and C as compare to control group; however, a significant decrease in egg weight was seen at 4th, 5th and 6th week in moldy feed treated group B, while non-significant difference was noted in combination groups C, D and E as compared to group A (Table.3). All the birds in group A remained active throughout the experiment, while group B remained depressed and had decreased attraction toward feed and water. The bird in group C, D and E showed some ameliorative effects in clinical signs and behavior as compared to Group B (Table 4). The relative weight of liver, kidney, heart and spleen increased significantly ($P < 0.05$) in group B and C as compared to group A (Table 5).

3.2 GROSS AND HISTOLOGICAL LESIONS

No gross lesions were observed in group A. Group B showed enlarged, friable, pale light color liver along with enlarged kidneys (Figure 1c) and ecchymotic hemorrhages (Figure 1a) were present on the surface of liver, kidneys, heart and intestines. Group C, D and E also showed similar lesions, but the severity of gross lesions in various organs gradually decreased due addition of different concentration of MTS in moldy feed (Figure 1b, 1d). At 6th week of the experiment, hepatocytes were normal in appearance, having nucleolus with fine chromatin material in group A. There was vacuolar degeneration of hepatocytes in group B (Figure 2a), while the liver of group D appeared to be normal (Figure 2b). Group C birds showed cellular infiltration and pyknotic nuclei in few areas of the liver. In group D, very few sinusoidal spaces were noticeable, along with cellular infiltration in few places, and hyperemia was also present. Renal parenchyma of birds from group A was normal with normal nuclei of tubular epithelial cells. Kidneys of group B showed pyknotic nuclei of tubular epithelial cells, indicating acute tubular necrosis, along with mild to moderate congestion in the renal parenchyma (Figure 2c). Kidneys of group C birds have mild congestion, but the nuclei of tubular epithelial cell were normal. Mild congestion in renal parenchyma was also seen in group D (Figure 2d). In group D, changes in liver and kidneys indicated partial amelioration of mycotoxins with MTS. In group E, liver and kidney parenchyma was normal, but in kidneys, urinary spaces in some places were not cleared indicating partial to complete amelioration of mycotoxins. The intestines in the control group were normal in appearance; however, intestines in birds of group B

exhibited mild ecchymotic hemorrhages on the mucosal surface, whereas such lesions were not present in birds of groups C to E.

3.3 HEMATOLOGICAL PARAMETER

Total erythrocyte count, total leukocyte count, Hb concentration and hematocrit were lowered significantly ($P < 0.05$) in group B as compared to group A. RBC indices i.e. MCV, MCH and MCHC varied non-significantly as compared to group A (Table 6).

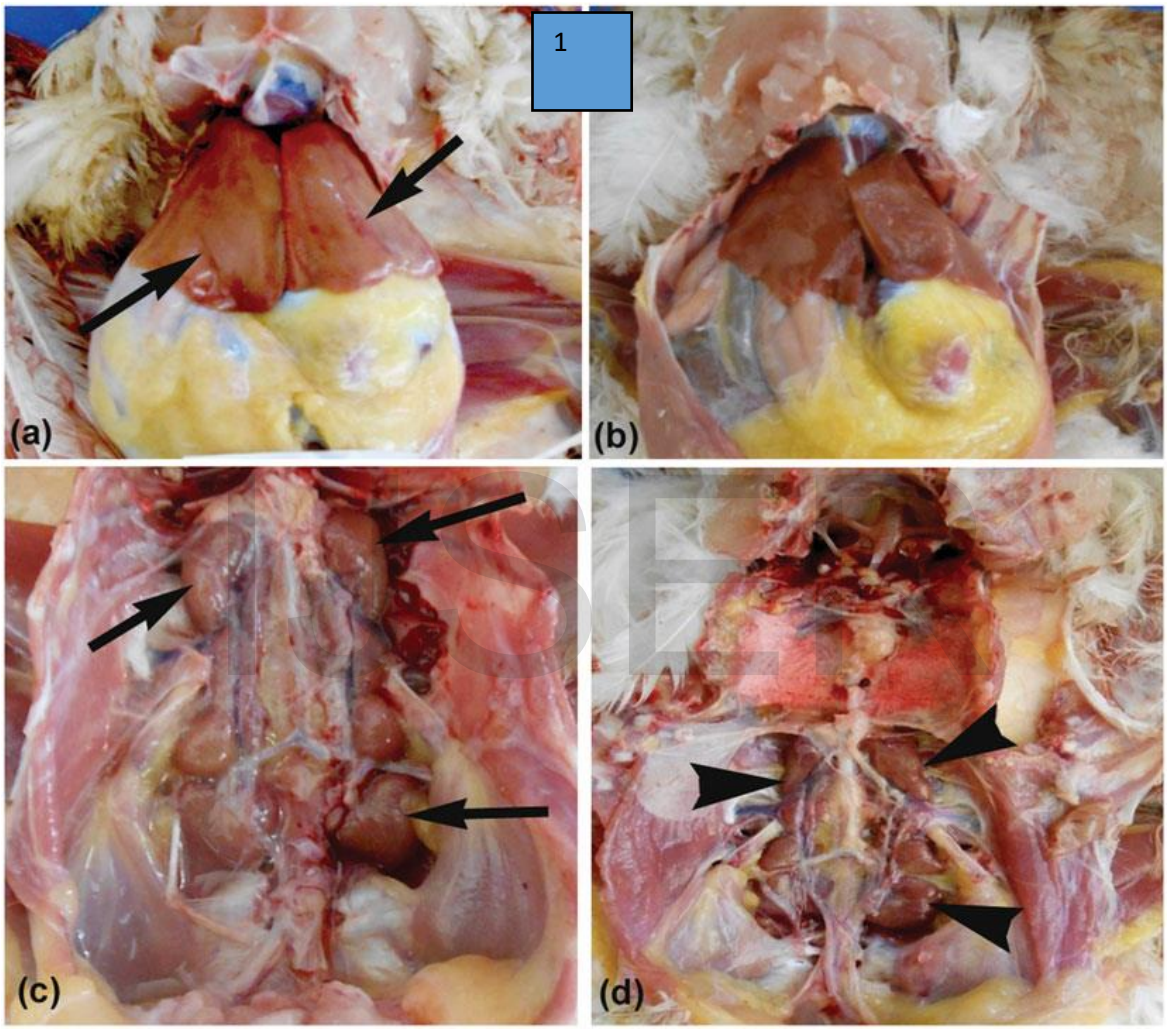

FIGURE 1. Gross lesions observed in liver and kidneys of various groups. (a) Hemorrhages on liver (arrows), (b) no lesion on liver, (c) swollen kidneys (arrows) and (d) less swollen kidneys (arrowheads). Birds of (a) and (c) were kept on moldy feed having no MTS while, birds of (b) and (d) were fed moldy feed along with 2% milk thistle seed.

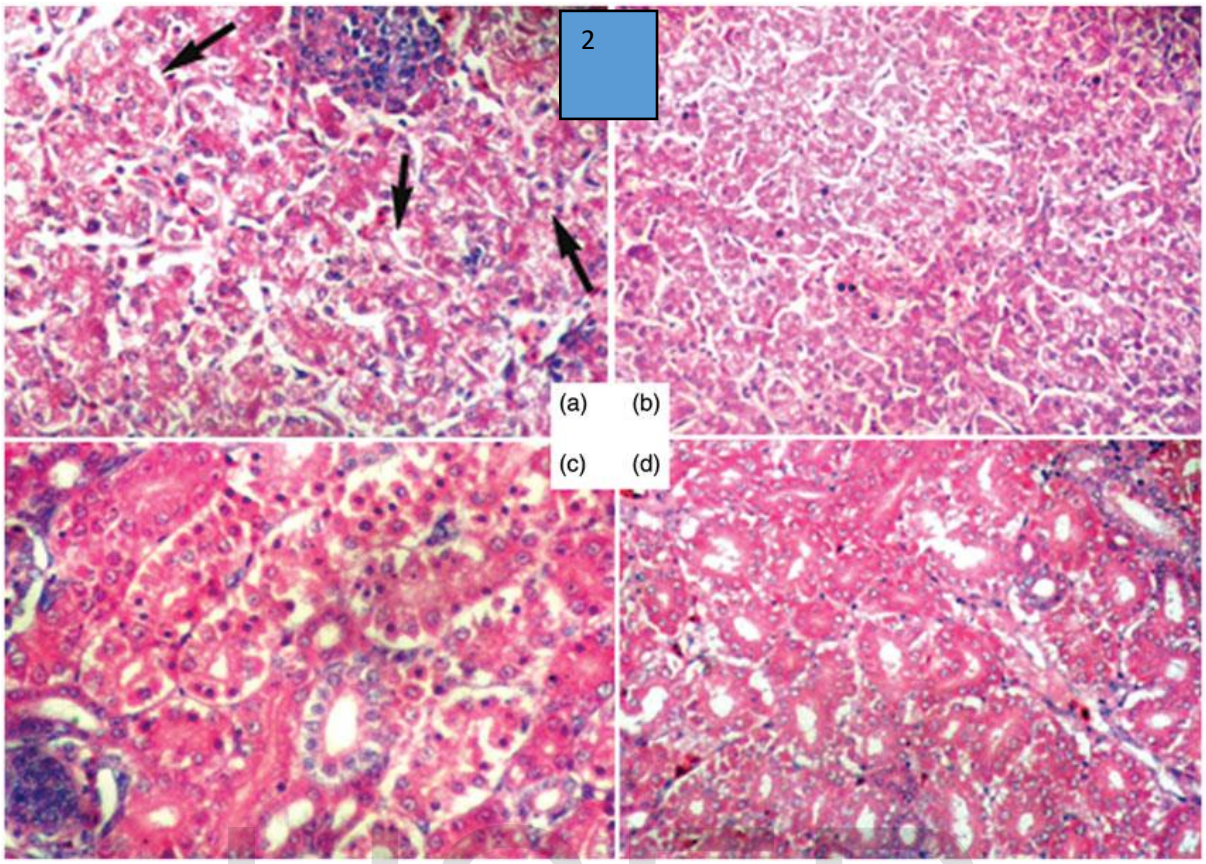

FIGURE 2. Photomicrograph of liver and kidneys of various groups H & E. 200. (a) Mild vacuolar degeneration (arrows), (b) No lesions seen in liver, (c) Pyknotic nuclei in tubular epithelial cells of kidneys (arrows) and (d) Normal renal parenchyma indication of amelioration of toxic effects. Birds of (a) and (c) were kept on moldy feed with no MTS while, birds of (b) and (d) were fed moldy feed along with 2% milk thistle seed.

TABLE 1: FEED INTAKE (G) OF VARIOUS GROUPS OFFERED MOLDY FEED ALONE AND WITH VARIOUS CONCENTRATIONS OF MILK THISTLE SEED FOR DIFFERENT WEEKS.

Group	Experimental					
	1	2	3	4	5	6
A	80.6 ± 8.5d	84.2±7.3ab	99.3 ± 7.3b	92.1 ± 7.7a	99.7 ± 9.4a	104.4 ± 4.6a
B	84.2 ± 7.3cd	76.4 ± 0.8b	79.2 ± 6.4c	83.7 ± 11.5b	86.6 ± 8.9b	87.3 ± 2.9c
C	99.9 ± 5.6ac	90.9 ± 6.3a	101.3± 12.9a	95.7 ± 5.1a	96.2 ± 8.2a	105.6 ± 4.9a
D	93.4 ± 7.6ab	85.7 ± 8.9a	105.6 ± 7.3a	95.4 ± 13.5a	98.3 ± 4.1a	104.0 ± 4.2a
E	90.2 ± 6.7bc	86.9 ± 5.5a	106.3 ± 4.7a	96.3 ± 11.6a	99.7 ± 3.7a	102.0 ± 2.6a

Values (Mean ± SD) in each column with different superscript varies from each other significantly at $P < 0.05$.

TABLE 2: BODY WEIGHTS (G) OF WHITE LEGHORN LAYERS OFFERED MOLDY FEED ALONE AND WITH VARIOUS CONCENTRATIONS OF MILK THISTLE SEED FOR DIFFERENT WEEKS.

Group	Day 0	Experimental Weeks					
		1	2	3	4	5	6
A	1752.0 ±43.7a	1792.8±11 3.4a	1792.8 ±113.4b	1792.8 ±113.4a	1792.8 ±113.4a	1792.8 ±63.4a	1607.2 ±148.4a
B	1749.5 ±31.0a	1542.8 ±78.7c	1542.8 ±78.7c	1542.8 ±78.7c	1542.8 ±78.7c	1542.8 ±78.7c	1475.7 ±60.2b
C	1712.5 ±51.9a	1778.6 ±197.6a	1778.6 ±197.6ab	1778.6 ±97.6ab	1778.6± 97.6ab	1778.6 ±197.6b	1478.6 ±107.5b
D	1741.3 ±23.9a	1635.7± 69.0bc	1671.4 ±118.5b	1650.0 ±57.7bc	1657.2±67 .3bc	1621.4 ±75.6bc	1524.3 ±124.4ab
E	1775.0 ±68.1a	1728.6 ±80.9ab	1685.7 ±114.4ab	1557.2 ±73.5c	1777.2±43 .2ab	1728.6 ±69.8b	1644.3 ±130.6a

Values (Mean ± SD) in each column with different superscript varies from each other significantly at $P < 0.05$.

TABLE 3: WEEKLY EGG PRODUCTION AND EGG WEIGHT OF VARIOUS GROUPS OFFERED MOLDY FEED ALONE AND WITH DIFFERENT CONCENTRATIONS OF MILK THISTLE SEED FOR DIFFERENT WEEKS.

Egg production (%) in various group	Experimental weeks					
	1	2	3	4	5	6
A	82 ± 3.7abc	80 ± 4.1ad	81.2 ± 6.5a	82.97±5.7a	80.9 ± 5.1a	81.1 ± 5.0a
B	80 ±4.5abc	77 ± 6.7dc	75.7 ± 3.2d	74 ± 3.11c	73.1 ± 2.9d	71.10 ± 6.7d
C	83 ± 5.7ab	80 ± 6.5b	78 ± 4.7c	79.1 ± 4.1d	80.7 ± 3.1ba	80 ± 5.5ba
D	84 ± 6.1a	82 ± 4.4a	80.7 ± 3.7ab	80.12±3.11ab	79.2 ± 3.0bc	78.1±3.9a
E	82 ± 3.2ac	80 ± 5.5ac	78.11± 3.9cb	78.6 ± 3.5ab	78.60 ± 4.1c	77.17 ± 3.6c
Egg weight (g)						
A	63.55 ±0.1a	63.24 ± 1.4a	60.43 ± 2.4a	62.77 ± 2.4a	62.74 ± 4.8a	60.74 ± 2.8a
B	62.91±.3a	61.88 ± 0.6a	58.76 ± 2.2b	58.67 ± 2.2a	57.58 ± 3.2b	58.48 ± 2.7b
C	63.12± 2.9a	61.42 ± 3.2a	60.20 ± 1.6b	60.91 ± 2.3a	59.79 ± 2.4a	61.42 ± 3.6a
D	62.76± 1.4a	62.01 ± 1.1a	61.33 ± 2.09a	60.49 ± 2.4a	61.48 ± 3.6a	63.11 ± 4.02a
E	61.31±2.2a	59.98 ± 3.8a	60.91 ± 1.9a	60.65 ± 1.5a	59.91 ± 2.10a	63.53 ± 4.2a

Values (Mean ± SD) in each column under a specific parameter followed by different superscripts are Significantly different at $P < 0.05$.

TABLE 4: SCORING OF CLINICAL SIGNS AND ALTERATIONS IN BEHAVIOR OF VARIOUS GROUPS OFFERED MOLDY FEED ALONE AND WITH VARIOUS CONCENTRATIONS OF MILK THISTLE SEEDS FOR DIFFERENT WEEKS

Week	Clinical signs/ Behavior	Score range	Experimental Groups				
			A	B	C	D	E
1	Alertness	0-4	4	4	3	4	4
	Feces consistency	0-4	4	3	3	4	4
	Feathers	0-4	4	3	3	4	4
	Total		12	10	9	12	12
2	Alertness	0-4	4	4	3	3	3
	Feces consistency	0-4	4	2	2	2	2

	Feathers	0-4	4	3	3	3	3
	Total		12	9	8	8	8
3	Alertness	0-4	4	2	3	3	4
	Feces consistency	0-4	4	2	2	3	3
	Feathers	0-4	4	2	3	3	3
	Total		12	6	8	9	10
4	Alertness	0-4	4	2	3	3	3
	Feces consistency	0-4	4	2	2	2	4
	Feathers	0-4	4	2	2	3	3
	Total		12	6	7	8	10
5	Alertness	0-4	4	2	2	3	3
	Feces consistency	0-4	4	2	2	2	3
	Feathers	0-4	4	1	2	2	4
	Total		12	5	6	7	10
6	Alertness	0-4	4	1	2	2	3
	Feces consistency	0-4	4	2	1	2	3
	Feathers	0-4	4	1	2	2	2
	Total		12	4	5	6	8
Grand total			72	37	43	48	54

TABLE 5: RELATIVE WEIGHT OF DIFFERENT ORGANS OF VARIOUS GROUPS OFFERED MOLDY FEED ALONE AND WITH VARIOUS CONCENTRATION OF MILK THISTLE SEEDS FOR DIFFERENT WEEKS.

Group	Liver	Kidneys	Heart	Spleen
A	2.44 ± 0.25b	0.74 ± 0.19b	0.60 ± 0.04a	0.11 ± 0.03a
B	2.92 ± 0.24a	0.86 ± 0.06a	0.74 ± 0.09b	0.17 ± 0.02b
C	2.25 ± 0.32b	0.72 ± 0.13ab	0.70 ± 0.05b	0.12 ± 0.03ab
D	2.06 ± 0.54b	0.64 ± 0.14b	0.65 ± 0.03ab	0.11 ± 0.03a
E	2.65 ± 0.05a	0.78 ± 0.08a	0.63 ± 0.05ab	0.09 ± 0.03a

Values (Mean ± SD) in each column with different superscript varies from each other significantly at $P < 0.05$.

TABLE 6: HEMATOLOGICAL VALUES OF VARIOUS GROUPS FEED MOLDY FEED ALONE AND WITH VARIOUS CONCENTRATION OF MILK THISTLE SEED.

Group	TEC (10 6 mL)	TLC (103)	Hb (g/dL)	Hematocrit (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
A	3.09±0.15a	26.38±1.07a	10.93±0.23a	30.83±0.69a	99.94±5.80	35.41±1.93	35.44±0.56
B	2.82±0.10b	22.96±0.95b	9.36±0.34b	27.33±0.39b	96.22±3.37	33.96±1.72	35.29±1.12
C	3.07±0.11a	24.31±0.52ab	10.97±0.31a	30.66±0.47a	99.96±3.99	35.77±1.73	35.79±1.17
D	3.05±0.12a	25.30±0.44ab	10.36±0.38a	30.50±0.50a	97.14±7.23	34.05±1.93	35.10±0.78
E	3.13±0.12a	26.28±0.34a	10.90±0.50a	30.90±0.68a	98.6±5.37	34.79±0.93	35.39±2.20

Values (Mean ± SD) in each column followed by different superscripts differ significantly at $P < 0.05$. MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

DISCUSSION

Poultry is one of the vibrant segment of agriculture in Pakistan, therefore, poultry feed production is increased due to high demand for poultry meat and eggs. For feed formulation different cereals are used. In Pakistan climatic conditions are hot and humid. Due to low standard storage facilities these cereals

become contaminated with mycotoxins that produce injurious effects in poultry and consumers as well (Murugesan et al., 2015; Robens and Richard, 1992). Mycotoxins and their toxic effects can be reduced by dietary incorporation of various substances which are chemical or biological in nature. Milk thistle is a locally existing medicinal herb found in Pakistan (Muhammad et al., 2012). The current study was designed to determine the toxic effects of moldy feed in commercial white Leghorn layers and their amelioration with milk thistle seed. All the birds in group A were active and revealed no clinical signs throughout the experimental trails, while birds in group B were depressed with ruffled feathers, anorexic, the consistency of feces was watery in most of the birds and the eggs produced were of thin and soft shelled. These investigations are in accordance with Khan et al. (2010) and Smith et al. (1975). The clinical signs in group C, D and E were less severe as compared to group B, similarly the mortality was comparatively higher in group B than other treated groups, which might be due to ameliorative effects of increase level of milk thistle seed in feed. Kumar et al. (2015) also, reported that mortality rate and clinical signs in poultry were significantly decreased in aflatoxicosis by addition of Silybum marianum in feed. The body weight gain in group C, D and E was higher than group B which is in accordance with the growth promoting effect observed by Muhammad et al. (2012) in poultry by using milk thistle in feed. Like the body weight feed intake was also significantly decreased in group B as compared to group C, D, and E these findings are similar with those previously perceived by Yunus et al. (2011) while, Ahmad et al. (2012) reported improvement in feed intake in aflotoxicosis by using milk thistle seeds in feed. The significant decrease in egg weight and production in group B is probably associated with reduced feed intake and decrease metabolism due to toxin burden in birds (Aly and Anwer, 2009; Abdelhamid and Dorra, 1990; Yegani et al., 2006). However, the improved quantity and quality of eggs produced in group C, D, and E is due to ameliorative effects of milk thistle (Levic et al., 2010). Several other experimental studies proved that the combined supplement of milk thistle seed improved productive and reproductive performances of breeding hens and turkeys due to the presence of linoleic acid, the essential nutrient for breeding flocks (Aydin and Cook, 2004; Levic et al., 2006; Levic et al. 2010; Aydin et al., 2001) The gross lesions observed in liver, kidneys, heart and spleen of group B and increase in relative weight of these organs are in accordance with the lesions previously reported by Khan et al. (2010) in birds suffering from aflotoxicosis. The decrease in severity of gross lesions in group C, D and E with addition of milk thistle in feed in a dose dependent manner shows the hepato protective and antitoxic effects of milk thistle. Ahmad et al. (2012) also, reported almost same hepato protective and other ameliorative effects of milk thistle in case of mycotoxicosis in layers. The microscopic lesion observed in birds of group B include vacuolar degeneration of hepatocytes, pyknotic nuclei with individual cell necrosis, heterophilic and mononuclear cellular infiltration, cell swelling and congestion of parenchyma in liver; congestion of renal parenchyma, necrosis and degeneration with pyknotic nuclei of tubular epithelial cells and cellular swelling of tubular epithelium in kidneys. Similar lesions were also, previously observed by in Valchev et al. (2014), Hussain et al. (2008) and Tessari et al. (2006). These lesions were less sever in group C, D and E. Khan et al. (2010) reported that Silybum marianum dietary addition helped to reduce aflatoxin induced histopathological alterations in various organs. Yunus et al. (2011) also reported the similar results. Total erythrocyte count, total leukocyte count, hemoglobin and hematocrit were significantly lower in group B, while a non-significant difference was noted in group C, D, and E as compared to group A. Significantly decreased red blood cell counts, Hb and hematocrit in aflatoxicosis could be due to the protein inhibiting effects of aflatoxins on hemopoietic organs (Kececi et al., 1998; Valchev et al., 2014). Sakhare et al. (2007) also reported a non-significant difference in TEC, TLC, Hb and RBC indices in aflotoxin effected birds treated with milk thistle seeds. It is concluded from the current study that addition of milk thistle seed in moldy feed with the concentration of 1 to 2 % has almost completely ameliorated the toxic effect of mycotoxicosis in poultry.

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