Effects of silymarin on productive performance, liver function and serum biochemical profile in broiler Japanese quail challenged with dietary aflatoxins

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ABSTRACT

A 2 × 3 factorial experiment was conducted to investigate the effects of feeding silymarin (0, 1000 and 2000 mg/kg) in aflatoxin contaminated diets (0 and 2.2 mg/kg) on selected performance, blood and liver parameters in broiler Japanese quails using 480 7-day-old mixed sex birds up to Day 35 of age. Feed intake, daily weight gain (DWG) and European production index (EPI) in Days 7–35 of age were reduced by 5.56, 5.97 and 10.97%, respectively, and mortality was increased in the birds fed on diets containing 2.2 mg/kg aflatoxin (p < .05). Mean ALT, alkaline phosphatase (ALK), uric acid in birds grown on aflatoxin contaminated diet were 28.32, 12.29 and 16.34% greater than those fed with control diet, respectively (p < .05). The birds fed with diets containing 1000 mg/kg silymarin showed greater DWG (6.35%) and EPI (12.89%) and lesser feed conversion ratio (6.6%) during Days 7–35 compared with control birds (p < .05). Mean ALT (37.46%), AST (16.90%) and ALK (27.67%) activity reduced in birds grown on diets containing 2000 mg/kg silymarin (p < .05). Mean serum concentration of phosphorous increased (13.44%) and glucose (GLU) decreased (10.37%) in the same birds compared with control quails (p < .05). A significant dietary silymarin × aflatoxin interaction observed for DWG, blood concentrations of ALT, AST, calcium, GLU, LDL, triglyceride and proportional weight of liver, testis and spleen in Day 35 of age. It was concluded that Supplementation of 1000 mg/kg silymarin into the contaminated diets alleviated the adverse impact of aflatoxins on bird’s performance.

HIGHLIGHTS

- Diets contaminated with aflatoxins cause impaired growth and altered hepatic function in broiler quails.
- Inclusion of silymarin in diet, alleviate the adverse impact of aflatoxins on bird’s performance.
- Greater levels of silymarin (2000 mg/kg) relieves the AF-induced intimidating alterations in the liver and blood parameters in quails.

Introduction

Natural diet contaminants including mycotoxins are among the main challenges for poultry men as well as quail producers (Kumar et al. 2017). Mycotoxins are worldwide found in all feed resources, mainly in corn, and cause huge economic losses through reduced birds health, immune response and performance (Giambrone et al. 1985; Yunus et al. 2011; Cheng et al. 2016; Pappas et al. 2016). Aflatoxins are rapidly absorbed throughout the gastrointestinal tract, spread all over the body (Sakamoto et al. 2017) and mainly metabolise in liver where they will be converted into the reactive and electrophilic entities by certain hepatic cytochrome enzymes (Wild and Montesano 2009; Wu and Khlangwiset 2010), the events which are indeed harmful to the liver cells and tissue integrity (Liu and Wu 2010). Liver is an important organ playing a key role in homeostasis (Sakamoto et al. 2017) through its broad enzymatic capability involving metabolism of carbohydrates, proteins, and lipids, immunity, de novo fat synthesis and the like (Thawley 2017). Liver functions exceed 2000 in the relevant literature. Grossly, liver health equals to a healthy metabolism, therefore liver protection against exogenous toxic and harmful substances has received a top

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priority in poultry research, particularly in fast-growing birds with boosted metabolic processes. Literature bears enormous recommendations on how mycotoxins effects in broiler industry can be alleviated, resulting in practical approaches and strategies to minimise the impacts of mycotoxins on growing birds (Zhu et al. 2016). The effects of adsorbents, organic acids, yeasts and sulphur amino acids on detoxification of mycotoxins in broiler diets have been widely studied (Santurio and sulphur amino acids on detoxification of mycotoxins (Santurio 2000; Santin et al. 2006). However, there is no worldwide agreement on effectiveness of all products because certain toxins, such as aflatoxins, are thermo-stable, resistant to some chemicals, soluble in polar solvents, and insoluble in fats and oils. Recently, administration of natural remedies into poultry diets has received huge attention to minimise toxic effects and impaired liver function in mycotoxins affected birds. It has been shown that certain compounds in herbal medicines are able to prevent lipid peroxidation in biological membranes and increase proliferation of new liver cells to replace the damaged ones (Kumar Jain et al. 2013). *Silybum marianum*, or milk thistle, is a well-known plant mainly interested for its hepatotonic extract named Silymarin. Silymarin contains various flavonolignans (silibinin, isosilibinin, silychristin, isosilychristin and silydianin) and a flavonoid (taxifolin) (Federico et al. 2017), which collectively exert anti-oxidative, anti-inflammatory, antifibrotic, antilipid peroxidative, cell membrane stabilising and liver regenerating effects. Silybin is usually the predominant active ingredient in silymarin, a substrate with antioxidant properties demonstrating hepatoprotective effects in human (Federico et al. 2017) as well as in the animal models (Madrigal-Santillan et al. 2014). Silymarin metabolically stimulates hepatic cells and activates the ribosomal RNA synthesis to stimulate protein formation (Vargas-Mendoza et al. 2014). Free radical scavenging and antioxidant properties of silymarin have attracted more attention in poultry research where they have demonstrated that silymarin restored the endogenous antioxidant enzymes (Superoxide dismutase, glutathione peroxidase and Catalase) and non-enzymatic antioxidants (vitamins E and C) in the liver of the stressed laying hens (Pradeep et al. 2007) and decreased lipid and protein oxidation in broiler chicken (Alhidary et al. 2017), decreased DNA fragmentation and apoptosis (Upadhyay et al. 2010), and reduced secretion of alamine aminotransferase (ALT) and aspartate aminotransferase (AST) from the liver into the plasma due to hepatic injuries caused by free radicals (Amiridumari et al. 2013; Sherif and Al-Gayyar 2013).

In view of the foresaid importance of detoxification or neutralisation of mycotoxins in poultry diets, this study aimed to further evaluation of feeding silymarin on productive performance, liver function and serum biochemical profile in broiler Japanese quail challenged with dietary aflatoxins.

### Material and methods

#### Experimental flock management

A total of 1200 Japanese quail (*Coturnix coturnix japonica*) were provided from a local hatchery and reared straight run in battery cages up to Day 7 of age. During the same period, ambient temperature and relative humidity were set at 38°C and 70%, respectively. Birds provided with a crumble diet containing 2900 kcal/kg metabolisable energy and 24% protein. On Day 7, 480 mixed sex birds with an

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Table 1. Ingredients and nutrient composition of the experimental diets for quail.

| Ingredient                  | Basal diet No aflatoxin | Experimental diet With aflatoxin |
|-----------------------------|-------------------------|---------------------------------|
| Ingredient, % as fed        |                         |                                 |
| Corn grain                  | 53.23                   | 49.82                           |
| Soybean meal                | 35.00                   | 35.00                           |
| Gluten meal                 | 7.60                    | 7.53                            |
| Rice                        | 0.00                    | 3.35                            |
| Calcium carbonate           | 1.37                    | 1.36                            |
| Dicalcium phosphate         | 0.97                    | 0.97                            |
| Sunflower oil               | 0.60                    | 0.76                            |
| Sodium chloride             | 0.26                    | 0.26                            |
| Sodium bicarbonate          | 0.13                    | 0.12                            |
| L-Lysine HCl – 79%          | 0.20                    | 0.19                            |
| L-Threonine                 | 0.14                    | 0.14                            |
| Mineral premix              | 0.25                    | 0.25                            |
| Vitamin premix              | 0.25                    | 0.25                            |
| Total                       | 100.00                  | 100.00                          |
| Calculated composition      |                         |                                 |
| Metabolizable energy (Kcal/kg) | 2900                  | 2900                            |
| Crude protein (%)           | 24.00                   | 24.00                           |
| Calcium (%)                 | 0.80                    | 0.80                            |
| Available phosphorus (%)    | 0.30                    | 0.30                            |
| Lysine (%)                  | 1.30                    | 1.30                            |
| Methionine (%)              | 0.50                    | 0.50                            |
| Methionine + Cysteine (%)   | 0.75                    | 0.75                            |
| Threonine (%)               | 1.02                    | 1.02                            |
| Tryptophan (%)              | 0.22                    | 0.22                            |
| Sodium (%)                  | 0.15                    | 0.15                            |
| Chloride (%)                | 0.14                    | 0.14                            |
| Potassium (%)               | 0.40                    | 0.40                            |
| DEB* (mEq/kg)               | 238                     | 235                             |
| Total aflatoxin (mg/kg)     | 0.00                    | 2.20                            |

*Data are means of four replicates.*
The average weight of 50±2 g were selected and transferred into 24 galvanised wire cages (70/C2×60/C2×30 cm) equipped with appropriate feeder and drinker and raised up to Day 35 of age. During the experimental period, house temperature was reduced by 2°C per every 3 days until received at 22°C, and then it was kept constant. Relative humidity was controlled on 55±10%. Experimental treatments consisted of six diets in a 2/C2×3 factorial arrangement viz. a corn-soy-bean meal basal diet with inclusion of silymarin (0, 1000 and 2000 mg/kg) and aflatoxins (0 and 2.2 mg/kg) or both. Effects of each diet were evaluated in 4 replicates of 20 birds each (\(n=80\) per treatment). Silymarin with a purity of 80.18% was provided from Zardband Pharmaceuticals co., Tehran, Iran. The experimental diets were formulated according to the recommendations of NRC (1994) (Table 1). The mineral and vitamin supplements were free of antibiotic and antioxidant substances. Birds were fed ad libitum throughout the rearing period on a 24-hour lightening schedule.

**Preparation of aflatoxins**

The aflatoxin mixture was produced by cultivating *Aspergillus parasiticus* NRRL 2999 through growing on rice grain, according to the methodology described by Shotwell et al. (1966), at the Laboratory of Food Microbiology and Mycotoxicology, Faculty of Animal Science, Zabol University, Iran. The concentration of aflatoxins in the contaminated rice samples was determined using a high performance liquid chromatography (HPLC) method (Shimadzu Corporation, Kyoto, Japan). The final aflatoxin mixture contained 68.19% AFB1; 4.57% AFB2; 24.96% AFG1; and 2.28% AFG2. The mixture was incorporated into diets to give a concentration of 2.2 mg/kg of aflatoxins, and the diets were stored throughout the experimental period in dark plastic buckets with lids.

**Data recording**

Data on live body weight and feed intake (FI) were collected weekly during the experimental period and data were used to calculate daily weight gain (DWG), FI, and feed conversion ratio (FCR). Mortality was recorded upon occurrence. European production index (EPI) was calculated based on the equation provided by Euribrid (1994); EPI= [(LW×S)/(FCR×AS)]×100, where LW is live weight (kg), S is survival rate (%), and AS is age of slaughter (day). The formula is used for calculation of the same index in broiler chicken.

**Serum biochemistry**

On Day 35 of the experiment, blood samples were collected by puncturing jugular vein in two male quails per experimental unit (8 birds/treatment; \(n=8\)). Subsequently, the samples were centrifuged at 3000×g for 10 min at 4°C and stored at −80°C pending biochemical analyses. Serum concentrations of biochemical constituents including certain enzymes ALT, AST and alkaline phosphatase (ALK), total protein (TP), albumin (ALB), uric acid (UA), creatinine (CRE), blood urea nitrogen, total bilirubin, direct bilirubin, indirect bilirubin, calcium (Ca), phosphorous (Pho), glucose (GLU), cholesterol (CHOL), low density lipoprotein (LDL), high density lipoprotein (HDL) and triglyceride (TG) were determined using an autoanalyser (UNIKON...
Table 3. Effects of silymarin in aflatoxin contaminated diets on feed intake of Japanese quail during Days 7–35 of age.

| Aflatoxin, mg/kg | Silymarin, mg/kg | Fl, g/day/bird |
|------------------|------------------|---------------|
|                  |                  | 7–14 d        | 15–21 d        | 22–28 d | 29–35 d | 7–21 d | 7–28 d | 7–35 d |
| 0                |                  | 14.8000       | 22.3000       | 25.9000 | 23.3000 | 18.5000 | 21.0000 | 21.6000 |
| 2.2              |                  | 14.6000 b     | 21.4000 b     | 24.4000 b | 21.3000 b | 18.0000 | 20.1000 b | 20.4000 b |
| SEM              |                  | 0.2000        | 0.3000        | 0.3000 | 0.3000 | 0.2000 | 0.2000 | 0.2000 |
| 0                | 0                | 14.7000       | 21.9000       | 25.2000 | 22.6000 | 18.3000 | 20.6000 | 21.1000 |
| 1000             | 14.6000         | 21.7000       | 24.9000       | 22.1000 | 18.1000 | 20.4000 | 20.8000 |
| 2000             | 14.7000         | 21.9000       | 25.3000       | 22.2000 | 18.3000 | 20.6000 | 21.0000 |
| SEM              | 0.3000          | 0.3000        | 0.4000        | 0.4000 | 0.2000 | 0.2000 | 0.2000 |
| 0                | 0                | 14.9000       | 22.3000       | 26.0000 | 23.8000 | 18.5000 | 21.0000 |
| 1000             | 14.6000         | 22.5000       | 26.0000       | 23.8000 | 18.5000 | 21.0000 |
| 2000             | 14.9000         | 21.9000       | 25.7000       | 22.5000 | 18.4000 | 20.8000 |
| SEM              | 0.3000          | 0.4000        | 0.5000        | 0.5000 | 0.6000 | 0.3000 |

Analysis of variance results

| Effect           | 0.9399 | 0.3478 | 0.4841 | 0.1012 | 0.6857 | 0.4622 | 0.1202 |
|------------------|--------|--------|--------|--------|--------|--------|--------|
| Aflatoxin        | 0.5427 | 0.0483 | 0.0043 | 0.0007 | 0.0730 | 0.0058 | 0.0002 |
| Silymarin        | 0.9136 | 0.8948 | 0.7516 | 0.6940 | 0.8836 | 0.7533 | 0.6925 |
| Interaction      | 0.9399 | 0.3478 | 0.4841 | 0.1012 | 0.6857 | 0.4622 | 0.1202 |

SEM Means within the same column with different letters are significantly different by Tukey test (p < .05).

Fl: Feed intake (g/day/bird); SEM: standard error of the mean (n = 80).

933; Kontron Co. Ltd., Milan, Italy). This analyser employs enzymatic procedures using SEPPIM Diagnostic Kits (SEPPIM S.A.S., Sees, France) in two replicates, at 25 °C, that have been described by Elliott (1984) and adopted by Khosravinia (2015). Sera globulin (GLOB) concentration was calculated by subtracting ALB from TP (GLOB = TP – ALB) (Sakamoto et al. 2017). At the end of the trial, two male quails from each experimental unit (8 birds/treatment; n = 8) were selected, weighted and killed by cervical dislocation and necropsied. Liver, pancreas, testis and spleen were separated and weighted.

Statistical analysis

This study was performed in a 2 × 3 factorial arrangement to investigate the fixed effects of silymarin in three levels (0, 1000 and 2000 mg/kg in diet), aflatoxins in two levels (0 and 2.2 mg/kg in diet) and their interactions. Experimental treatments were examined in four replicates of 20 birds each. A completely randomised design was used to evaluate the response of broiler quails to the six experimental treatments. All data were analysed using PROC GLM in Statistical Analysis System, version 9.1 (SAS Institute 2002). The Tukey’s test was used to differentiate the multiple treatment means. For all tests, significance was declared at 5% (p > .05).

Results

The FI, DWG and EPI were reduced by 1.2g (5.56%), 0.4g (5.97%) and 2.3 unit (10.97%) respectively, and mortality was increased in the birds fed on diets containing 2.2 mg/kg aflatoxin during Days 7–35 of age (p < .05; Table 2). Mean ALT and ALK for birds grown on aflatoxin contaminated diet were 4U/L (28.32%) and 190.833U/L (12.29%) greater than those fed with control diet (p < .05; Table 3). The birds fed with diets containing 2.2 mg/kg aflatoxin showed lesser TP (0.241g/dL or 16.34%) compared with those given uncontaminated diet at Day 35 of age (p < .05; Table 4). Mean CHOL, LDL and HDL in the birds receiving aflatoxin contaminated diet were 39.958 (20.29%), 33.275 (38.65%) and 23.458mg/dL (21.36%) lesser than those fed with control diet at the end of experimental period (p < .05; Table 5). Testis proportional weight decreased and liver as well as spleen percentage increased in the birds fed with diets containing 2.2 mg/kg aflatoxin at Day 35 of age (p < .05; Table 6).

Greater EPI by 2.41 unit (12.89%) achieved in the birds maintained on diets supplemented with 1000 mg/kg silymarin compared with control birds (p < .05; Table 2). Increased DWG (0.4g or 6.35%) were also observed in the same birds compared with control quails in 7–35 days (p < .05; Table 2). Mean FCR was lesser (0.22 unit or 6.6%) in the same birds during Days 7–35 compared with control birds (p < .05; Table 2). The birds received diets containing 2000 mg/kg silymarin had lesser serum ALT (7.75U/L or 37.46%), AST (36.5U/L or 16.90%) and ALK (397.188U/L or 27.67%) activity at the close of the experiment (p < .05; Table 3). Mean serum concentration of Pho increased (0.95mg/dL or 13.44%) and GLU decreased (34.44mg/dL or 10.37%) in the same birds compared with control quails (p < .05; Table 4). Lowered serum concentrations of LDL (36.03mg/dL or 40.6%)...
and TG (38.63 mg/dL or 33.77%) observed in the birds grown on diets containing 1000 mg/kg silymarin at day 35 of age (\(p < .05\); Table 5). The birds fed with diets containing silymarin had lesser liver and spleen percentage and greater proportional testis weight (\(p < .05\); Table 6).

A significant interaction between dietary silymarin and aflatoxin was observed for DWG in 7–35 days of age (Table 2), where birds maintained on uncontaminated diets containing 1000 mg silymarin demonstrated greater daily gain compared with other birds. Mean serum activity of ALT, AST (Table 3) and concentration of Ca, GLU (Table 4), HDL and TG (Table 5) were affected by dietary silymarin × dietary aflatoxin interaction in Day 35 of age. The proportional weight of liver, testis and spleen were significantly altered by interaction between the fixed effects considered at the same age (Table 6).

**Discussion**

The adverse effects of aflatoxin on bird’s performance and health have been considered in research since the early development of the poultry industry. Huge number of reports have been appeared in literature demonstrating decreased FI, DWG and FCR in broiler chicken (Tedesco et al. 2004; Del Bianchi et al. 2005; Yunus et al. 2011) turkey (Quist et al. 2000; Rauber et al. 2007), ducks (He et al. 2013; Chen et al. 2014; Chang et al. 2016) and

### Table 4. Effects of Silymarin in aflatoxin contaminated diets on daily weight gain of Japanese quail during Days 7–35 of age.

| Aflatoxin, mg/kg | Silymarin, mg/kg | 7–14 d | 15–21 d | 22–28 d | 29–35 d | 7–21 d | 7–28 d | 7–35 d |
|-----------------|-----------------|--------|--------|--------|--------|--------|--------|--------|
| 0               | 7.3000          | 3.9000 | 7.7000 | 6.7000 |
| 2.2             | 6.9000          | 3.5000 | 7.5000 | 6.3000 |
| SEM             | 0.0000          | 0.2000 | 0.0000 | 0.0000 |

### Table 5. Effects of silymarin in aflatoxin contaminated diets on feed conversion ratio of Japanese quail during Days 7–35 of age.

| Aflatoxin, mg/kg | Silymarin, mg/kg | 7–14 d | 15–21 d | 22–28 d | 29–35 d | 7–21 d | 7–28 d | 7–35 d |
|-----------------|-----------------|--------|--------|--------|--------|--------|--------|--------|
| 0               | 2.0200          | 6.0900 | 2.3300 | 2.7400 | 3.2200 |
| 2.2             | 2.1100          | 6.3600 | 2.3900 | 2.7900 | 3.2500 |
| SEM             | 0.0400          | 0.3100 | 0.0300 | 0.0300 | 0.0300 |

### Analysis of variance results

- **Aflatoxin**: <0.0001 0.4300 0.6181 0.5468 0.0987 0.2341 0.4446
- **Silymarin**: 0.2045 0.2104 0.1296 0.7828 0.0612 0.0085 0.0016
- **Interaction**: 0.1058 0.7648 0.2103 0.6467 0.2081 0.2015

### Notes

-Means within the same column with different letters are significantly different by Tukey test (\(p < .05\)).

-DWG: Daily weight gain (g/day/bird); SEM: standard error of the mean (\(n = 80\)).

-FCR: Feed conversion ratio (g:g); SEM: standard error of the mean (\(n = 80\)).
quail (Oliveira et al. 2002; Bagherzadeh and Mehri 2015) following feeding aflatoxin contaminated diets. Outcomes of the current study in the line with almost all previous finding confirmed decreased FI, DWG and EPI in quails fed with aflatoxin contaminated diets. Oliveira et al. (2002) reported decreased FI in 7-week-old quails fed with aflatoxin contaminated diets. Oliveira’s previous finding confirmed decreased FI, DWG and EPI in quails following feeding aflatoxin contaminated diets. Likewise, dietary inclusions of AF at 0, 1000 and 2000 μg/kg for 168 days. Similarly, Japanese quail fed diets containing 50 and 100 μg AF/kg specifically at its highest dose had an adverse effect on FI (Abreu et al. 2008). Similarly, Aflatoxin, mg/kg

Table 6. Effects of silymarin in aflatoxin contaminated diets on hepatic enzyme concentrations of Japanese quail during Days 7–35 of age.

| Aflatoxin, mg/kg | Silymarin, mg/kg | ALT, U/L | AST, U/L | ALK, U/L |
|-----------------|-----------------|----------|----------|----------|
| 0               | 14.125³         | 6.028    | 0.976    | 0.0094   |
| 2.2             | 18.125⁰         | 6.325    | 0.967    | 0.0096   |
| SEM             | 0.9720          | 4.7540   | 0.5719   | 0.0089   |

Analysis of variance results

| Aflatoxin | Silymarin | ALT, U/L | AST, U/L | ALK, U/L |
|-----------|-----------|----------|----------|----------|
| 0         | 2.7540²   | 6.028    | 0.976    | 0.0094   |
| 2.2       | 2.5130²   | 6.325    | 0.967    | 0.0096   |
| SEM       | 0.0760    | 0.0430   | 0.0710   | 0.0089   |

Table 7. Effects of silymarin in aflatoxin contaminated diets on serum biochemical profile concentrations of Japanese quail during Days 7–35 of age.

| Aflatoxin, mg/kg | Silymarin, mg/kg | TP, g/dL | GLO, g/dL | ALB, g/dL | ALB:GLO | BUN, mg/dL | CRE, mg/dL | UA, mg/dL | Ca, mg/dL | Pho, mg/dL | GLU, mg/dL |
|-----------------|-----------------|----------|----------|----------|----------|------------|------------|----------|----------|-----------|-----------|
| 0               | 2.7540²         | 1.0600   | 1.7480   | 1.8170   | 2.1000   | 0.3880     | 0.0094     |
| 2.2             | 2.5130²         | 0.8900   | 1.6230   | 1.9040   | 1.8230   | 0.3300     | 0.0094     |
| SEM             | 0.0760          | 0.0430   | 0.0710   | 0.0100   | 0.1610   | 0.0100     | 0.0094     |

Analysis of variance results

| Aflatoxin | Silymarin | ALT, U/L | AST, U/L | ALK, U/L |
|-----------|-----------|----------|----------|----------|
| 0         | 2.7540²   | 6.028    | 0.976    | 0.0094   |
| 2.2       | 2.5130²   | 6.325    | 0.967    | 0.0096   |
| SEM       | 0.0760    | 0.0430   | 0.0710   | 0.0089   |

[^2]: Means within the same column with different letters are significantly different by Tukey test (p < .05).

[^3]: ALT: Alanine amino transferase (U/L); AST: Aspartate amino transferase (U/L); ALK: Alkaline phosphatase (U/L); SEM: standard error of the mean (n = 8).
concentrations of ALK and ALT were observed in quails fed aflatoxins, which was an indication of liver injury (Campbell and Coles 1986; Gowda et al. 2008). ALT is predominantly found in the liver with negligible quantities in the kidney, heart, and skeletal muscles, while AST is found in liver and non-hepatic tissues including cardiac muscle, skeletal muscles, kidneys, brain, and red blood cells. As a result, ALT is a more specific indicator of liver damage than AST. While liver plays a central role in metabolism, altered concentrations for many serum biochemical components are anticipated by feeding quails chicken with AF contaminated diets as observed in the current study (Table 7).

Finally, aflatoxins like all other toxicants cause anorexia (Sehu et al. 2005; Yunus et al. 2011) through influencing central appetite modulating mechanisms (Sakamoto et al. 2017), resulting in a lesser DWG which was also observed in our study. Results of the current study agree those from Quist et al. (2000), Deanicke et al. (2003) and Denli et al. (2009) whereas disagree Politis et al. (2005) and Awad et al. (2006) reports who announced no remarkable impact for mycotoxin challenge on growth performance in broiler chicks.

Poultry industry continuously acquired commercial feed additive products to alleviate mycotoxins in diets. To date, many mycotoxin absorbents in physical, chemical and biological categories have been introduced and adopted widely in poultry nutrition (Ismail et al. 2018). Recent focus on herbal remedies has

| Table 8. Effects of silymarin in aflatoxin contaminated diets on serum concentration of bilirubin and lipid profile concentrations of Japanese quail during Days 7–35 of age. |
|---------------------------------|-----------------|-----------------|-----------------|---------------|-----------------|---------------|-----------------|
| Aflatoxin, mg/kg | Silymarin, mg/kg | CHOL, mg/dL | LDL, mg/dL | HDL, mg/dL | TG, mg/dL | TB, mg/dL | DB, mg/dL | IB, mg/dL |
| 0 | 196.9580 | 86.0920 | 109.8330 | 98.7500 | 0.2030 | 0.0330 | 0.1700 |
| 2.2 | 157.0000 | 52.8170 | 86.3750 | 89.0830 | 0.1960 | 0.0330 | 0.1630 |
| SEM | 8.7400 | 7.1020 | 4.9740 | 5.1970 | 0.0100 | 0.0030 | 0.0080 |
| 0 | 181.1880 | 89.9380 | 114.3750 | 105.8750 | 0.1940 | 0.0330 | 0.1640 |
| 1000 | 179.6880 | 64.5130 | 94.4380 | 91.6250 | 0.1860 | 0.0310 | 0.1550 |
| 2000 | 172.7500 | 53.2000 | 117.8750 | 75.7500 | 0.2110 | 0.0340 | 0.1780 |
| SEM | 10.7040 | 8.6990 | 6.0920 | 6.3650 | 0.0120 | 0.0030 | 0.0100 |
| 0 | 221.0000 | 135.3750 | 106.7500 | 150.6250 | 0.2100 | 0.0350 | 0.1750 |
| 0 | 170.0630 | 52.8170 | 86.3750 | 89.0830 | 0.1960 | 0.0330 | 0.1790 |
| SEM | 0.3 | 15.1380 | 12.3020 | 8.6160 | 9.0020 | 0.0170 | 0.0050 |

Analysis of variance results

**Aflatoxin** | 0.0046 | 0.0136 | 0.0137 | 0.6211 | 1.0000 | 0.5370 |

**Silymarin** | 0.7016 | 0.0255 | 0.3181 | 0.0017 | 0.2849 | 0.6548 |

**Interaction** | 0.0723 | 0.0027 | 0.9633 | <0.0001 | 0.6528 | 0.6681 |

Means within the same column with different letters are significantly different by Tukey test (p<.05).

CHOL: Cholesterol (mg/dL); LDL: Low density lipoprotein (mg/dL); HDL: High density lipoprotein (mg/dL); TG: Triglyceride (mg/dL); TB: Total bilirubin (mg/dL); DB: Direct bilirubin (mg/dL); IB: Indirect bilirubin (mg/dL); SEM: standard error of the mean (n=8).

| Table 9. Effects of silymarin in aflatoxin contaminated diets on relative weight of internal organs of Japanese quail during Days 7–35 of age. |
|---------------------------------|-----------------|-----------------|-----------------|---------------|
| Aflatoxin, mg/kg | Silymarin, mg/kg | Liver, % | Testis, % | Pancreas, % |
| 0 | 2.1580 | 1.0630 | 0.2660 | 0.0990 |
| 2.2 | 2.5250 | 1.0400 | 0.2730 | 0.1290 |
| SEM | 0.0230 | 0.0280 | 0.0120 | 0.0020 |
| 0 | 1.8020 | 1.3230 | 0.2420 | 0.0630 |
| 1000 | 1.8870 | 1.4830 | 0.2340 | 0.0820 |
| 2000 | 0.0280 | 0.0340 | 0.0140 | 0.0030 |
| 0 | 2.1100 | 1.4120 | 0.2480 | 0.1060 |
| 0 | 1.7910 | 1.5610 | 0.2330 | 0.0740 |
| 0 | 1.7050 | 1.3310 | 0.2190 | 0.0710 |
| 2.2 | 0 | 2.5930 | 0.6670 | 0.2970 | 0.1520 |
| 2.2 | 1.8130 | 1.0840 | 0.2510 | 0.0520 |
| 2.2 | 2.0690 | 1.4360 | 0.2490 | 0.0920 |
| SEM | 0.3000 | 0.0390 | 0.0480 | 0.0200 |

Analysis of variance results

**Aflatoxin** | <.0001 | <.0001 | <.0001 |

**Silymarin** | <.0001 | <.0001 | <.0001 |

**Interaction** | <.0001 | <.0001 | <.0001 |

Means within the same column with different letters are significantly different by Tukey test (p<.05).

SEM: standard error of the mean (n=8).
created a new hope for safe, effective and inexpensive substances demonstrating hepatoprotective effects against the harmful impact of mycotoxins on bird’s metabolism in particular on liver function and health. Milk thistle (*Silybum marianum*) extract known as silymarin has received huge attention in biological research. In the current study, greater EPI and improved DWG and FCR and lowered ALT, AST and ALK activity were observed in the birds received diets containing 1000 and 2000 mg/kg silymarin which was in agreement with findings of Neshatgharamaleki and Mohajeri (2014). Induced insulin secretion by pancreatic beta cells of the pancreas (Soto et al. 2004), promoted repair and renovation of the pancreatic tissue, protecting pancreatic tissue against damaging elements, thereby exerting hypoglycaemic effect are among mechanisms pointed out for silymarin beneficial effects on quail performance and health (Soto et al. 2003; Behboodi et al. 2017). Jahanian et al. (2017), using broiler chicks (7–28 days) reported that increasing dietary aflatoxin levels resulted in decreased FI and DWG, and compromised FCR in broiler chicks (7–28 days). We found that dietary supplementation with silymarin ameliorated the decreased FI and DWG, and improved FCR in aflatoxin-challenged chicks.

Considering all our results, diets contaminated with 2.2 mg/kg aflatoxin exerted adverse effects on quail’s performance as indicated by reduced FI, DWG and EPI, increased FCR and mortality and altered concentration of certain liver and blood parameters. Further indications were found where the same birds showed unfavourably alterations in concentration of certain liver and blood parameters. We found that dietary supplementation with 1000 mg/kg silymarin alleviated the hazardous effects of aflatoxin as evidenced by greater EPI, DWG, FI and improved FCR and in the birds fed AF contaminated diets. However greater levels of silymarin were needed to alleviate the impaired and blood biochemical parameters as reflected in blood concentrations of ALT, AST, Ca, GLU, HDL and TG in Day 35 of age and proportional weight of liver, testis and spleen in day 35 of age (Tables 8 and 9).

**Conclusions**

Feeding diets contaminated with 2.2 mg aflatoxins/kg impaired growth performance and altered hepatic function in broiler quails. Supplementation of 1000 mg/kg silymarin in the contaminated diets alleviated the adverse effects of aflatoxins on bird’s performance. Greater levels of silymarin (2000 mg/kg) were needed to relive the AF-induced intimidating alterations in the liver and blood parameters studied.

**Ethical approval**

All procedures carried out in this experiment were reviewed and approved by the Animal Care and Use Committee of Lorestan University, Khorramabad, Iran.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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