Efficacy of silymarin-nanohydrogle complex in attenuation of aflatoxins toxicity in Japanese quails

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ABSTRACT
A 2 × 3 factorial experiment was conducted to investigate the effects of silymarin-nanohydrogle (0, 500 free; 500 nano) and a mycotoxin (0; 2.2 mg/kg) silymarin contaminated diet on productive performance and certain serum biochemical parameters using 72 Japanese quail chicks in days 7–35 of age. Six experimental treatments consisting inclusion of 0 or 2.2 mg/kg aflatoxins in a basal diet fed to the birds receiving 0 or 500 mg/L silymarin in two free- or nanohydrogle forms via drinking water. Daily weight gain (DWG) and European production index (EPI) reduced by 6.7% and 13.6% while feed intake (FI) and FCR increased by 3.76% and 12% in the birds fed on the diet containing 2.2 mg/kg aflatoxin, respectively (p < .05). Administration of silymarin-nanohydrogle through drinking water improved FI and DWG by 3.7% and 8.1%, respectively (p < .05). Mean serum alkaline phosphatase (ALP) activity elevated by 26.1% and serum concentration of total protein (TP), glucose (GLU) and high density lipoproteins (HDL) declined by 14.4%, 6.1% and 27.1% in the birds fed on the aflatoxin-contaminated diet, respectively (p < .05). Mean serum concentration of BUN (86.4%) and GLU (12.0%) increased and Ca (10.3%) decreased in birds receiving 500 mg/kg silymarin-nanohydrogle (p < .05). The birds receiving silymarin-nanohydrogle in drinking water showed lesser liver and spleen percentage (p < .05). It was concluded that inclusion of 500 mg/kg silymarin-nanohydrogle in drinking water could significantly compensate the impaired growth performance and alter hepatic function in Japanese quails fed on a diet containing 2.2 mg aflatoxins.

HIGHLIGHTS
• Feeding diets contaminated with 2.2 mg aflatoxins/kg suppressed growth performance and impaired hepatic function in Japanese quails.
• Inclusion of 500 mg/kg silymarin-nanohydrogle in drinking water alleviated the adverse impact of aflatoxins on broilers’ performance and partially improved liver function.
• Greater levels of silymarin-nanohydrogle may relieve the aflatoxin-caused intimidating alterations in liver and blood parameters in Japanese quails.

Introduction
Mycotoxins impose massive economic losses in poultry industry worldwide through reducing bird’s health, immune response and production performance (Chang et al. 2016; Kumar et al. 2016; Khaleghipour et al. 2019). Effects of mycotoxins on animals comprise a wide range of pathological manifestations including hepatotoxicity, nephrotoxicity, immune suppression, oncogenesis and genotoxicity (Mishhairabgwi et al. 2019). Aflatoxins are fat soluble molecules rapidly absorbed throughout the gastrointestinal tract (Sakamoto et al. 2018) and mainly metabolised by the act of P450 cytochrome enzymes in liver, where they convert into destructive and electrophilic metabolites (Wu and Khlangwiset 2010). Therefore, protection of liver against exogenous toxic and harmful substances has received a top priority in poultry production, resulting in massive recommendations on attenuation of mycotoxins’ effects on broiler birds and productivity. For instances, the effects of adsorbents, organic acids, yeasts and sulphur amino acids on detoxification of mycotoxins in broiler diets are well addressed (Santurio 2000; Santin et al. 2006). Recently, using natural remedies poultry diets have received huge
Table 1. Ingredients and nutrient composition of the experimental diets for quail.

| Ingredient (% as fed) | Basal diet | Experimental diet |
|----------------------|------------|------------------|
|                      | No aflatoxin | With aflatoxin   |
| 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         | 0.20       | 0.19             |
| L-threonine          | 0.14       | 0.14             |
| Mineral premixb      | 0.25       | 0.25             |
| Vitamin premixc      | 0.25       | 0.25             |
| Total                | 100.00     | 100.00           |

Calculated composition

| Metabolisable 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 |
| DEBc (mEq/kg)                  | 238.00| 235.00|
| Total aflatoxin (mg/kg)        | 0.00 | 2.20 |

aRice was contaminated with aflatoxins; 65.7 mg/kg.
bMineral premix provided per kilogram of diet: Mn (from MnSO₄·H₂O), 65 mg; Zn (from ZnO), 55 mg; Fe (from FeSO₄·7H₂O), 50 mg; Cu (from CuSO₄·SH₂O), 8 mg; I (from Ca (IO₃)·2H₂O), 1.8 mg; Se, 0.30 mg; Co (fromCo₃O₄), 0.20 mg; Mo, 0.16 mg.
cVitamin premix provided per kilogram of diet: vitamin A (from vitamin A acetate), 11,500 U; cholecalciferol, 2100 U; vitamin E (from dl-α-tocopheryl acetate), 22 U; vitamin B₁₂, 0.60 mg; riboflavin, 4.4 mg; nicotinamide, 40 mg; calcium pantothenate, 35 mg; menadione (from menadione dimethyl-pyrimidinol), 1.50 mg; folic acid, 0.80 mg; thiamine, 3 mg; pyridoxine, 10 mg; biotin, 1 mg; choline chloride, 560 mg; ethoxyquin, 125 mg.
dDietary electrolyte balance: represents dietary Na + K – Cl in mEq/kg of diet.

attention as a promising strategy to minimise impaired liver function in the mycotoxins-affected birds.

In this contest, silymarin, the whole extract of milk thistle (Silybum marianum), is probably the most famous and well-investigated plant-derived feed additive. Silymarin contains various flavonolignans and flavono-oid (taxifolin) (Federico et al. 2017). Silymarin metabolically stimulates hepatic cells and activates the ribosomal RNA synthesis to stimulate protein formation (Vargas-Mendoza et al. 2014). It was shown that silymarin restored endogenous antioxidant enzymes and non-enzymatic antioxidants (vitamins E and C) in the liver of stressed laying hens (Pradeep et al. 2007) and diminished lipid and protein oxidation in broiler chickens (Alhidary et al. 2017). Reports also verified the reduced secretion of alanine aminotransferase and aspartate aminotransferase from the liver of the experimentally toxified animals into the plasma due to hepatic injuries resulting from free radicals (Sherif and Al-Gayyar 2013).

However, silymarin is almost water insoluble (0.04 mg/mL) (Javed et al. 2011) and absorbed from gastrointestinal tract in a minute rate (Wu et al. 2007). These facts necessitate research for incorporation of silymarin into compounds with a greater bioavailability for its active ingredients. A number of approaches including complexes of silymarin with microspheres (Abrol et al. 2004), liposomes (Yan-yyu et al. 2006), phospholipids (Kidd 2009), β-cyclodextrins (Voinovich et al. 2009) and nanocarriers (El-Sherbiny et al. 2011; Parveen et al. 2011) have been tried with inconsistent outcomes. An innovative approach to improve the bioavailability of silymarin may be reducing its particles to micro and nano sizes, which can result in greater drug efficacy. Micronisation and nanonisation are techniques to improve dissolution rate as well as bioavailability of a drug by reducing its particle size to 2–5 μm and 5–300 nm, respectively (Debbage 2009; Zhang et al. 2009). Therefore, this study aimed to further evaluate the effects of dietary inclusion of silymarin-nanohydrogel on production performance and selected serum biochemical factors in broiler Japanese quails challenged with dietary aflatoxins.

Material and methods

Experimental flock management

A total of 200 Japanese quails (Coturnix coturnix japonica) were provided from a local hatchery and reared straight run in battery cages up to day 7 of age. During this pre-experimentation period, ambient temperature and relative humidity were set at 38°C and 70%, respectively. Birds were fed with a crumble diet containing 2900 kcal/kg metabolisable energy and 24% protein. On day 7, 72 birds with an average weight of 55 ± 2 g were selected and transferred into 24 galvanised wire cages (50 × 50 × 30 cm) where they were used to examine the effects of six experimental treatments up to day 35 of age. During the experimental period, house temperature was reduced by 2°C per 3 days until it reached to 22°C and kept constant afterward. Relative humidity was controlled on 55 ± 10%. Experimental treatments consisted of six diets in a 2 × 3 factorial arrangement including a basal diet with and without inclusion of 500 mg silymarin in either free- or nano-hydrogel form in 1 L of drinking water provided to the birds fed on diets without or with aflatoxins contamination (0 or 2.2 mg/kg). Effects of each treatment were evaluated in four replicates of three birds each (n = 12 per treatment). The
experimental diets were formulated according to National Research Council (1994) (Table 1). The mineral and vitamin supplements were free of antibiotic and antioxidant substances. Birds were fed ad libitum throughout the experimental periods under a 24-h lightening schedule.

**Preparation of aflatoxin, silymarin and silymarin-nanohydrogel**

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). 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 aflatoxins/kg of diet. The diets were stored throughout the experimental period in dark glass bottle with lid and shaken strongly before administration. The silymarin-nanohydrogel complex (nano form, watery solution) containing 86.6% silymarin with a purity of 80.18% was prepared based on the methodology described by Lay Hong et al. (2013) in Zhinteb Knowledge Enterprise Co., Esfahan, Iran. Silymarin-nanohydrogel complex was incorporated into drinking water to give a concentration of 500 mg silymarin-nanohydrogel/kg of drinking water (500 ppm), and was stored all over the experimental period in dark glass bottle with lid. The nanoparticles were characterised in terms of size (expressed as average diameter) and zeta potential using a Zeta Sizer Nano Series® (Malvern Instruments, UK) equipped with a 4 mV He–Ne laser at a wavelength of 633 nm. Dynamic laser scattering at a scattering angle of 173° was used to measure the hydrodynamic diameter. A Laser Doppler Anemometry was applied to determine zeta potential in millivolts (mV) of the silymarin-nanohydrogel particles. The average size and mean zeta potential of silymarin-nanohydrogel particles were 127 nm (±6.35) and +18.43 mV (±0.92), respectively. The silymarin-nanohydrogel particles morphology (Figure 1) and surface topography were perceived microscopically with a Field Emission Scanning Electron Microscope (FESEM), (Model Quanta 400F, FEI Company, USA) at charge voltage of 3 kV.

**Data recording**

Data on live body weight (BW) and feed intake (FI) were collected weekly during the experimental period and data were used to calculate daily weight gain (DWG) 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 (%), FCR is feed conversion ratio and AS is age of slaughter (day).

**Serum biochemistry**

On day 35 of the experiment, blood samples were collected by puncturing jugular vein in four male quails per experimental treatment (four birds/treatment; n = 4). Subsequently, the samples were centrifuged at 3000 xg for 10 min at 4°C and stored at −80°C pending biochemical analyses. Serum concentrations of biochemical constituents including alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), gama-glutamyl
transaminase (GGT) and alkaline phosphatase (ALP), total protein (TP), albumin (ALB), uric acid (UA), creatinine (CRE), blood urea nitrogen (BUN), total bilirubin (TB), conjugated or direct bilirubin (DB), indirect bilirubin (IB), calcium (Ca), phosphorous (Pho), glucose (GLU), cholesterol (CHOL), low density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides (TG) were determined using an autoanalyser (UNIKON 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, adopted by Khosravinia (2015).

Sera globulin (GLOB) concentration was calculated by subtracting albumin from total protein (GLOB = TP – ALB) (Sakamoto et al. 2018). At the end of the trial, four male quails from each experimental treatment were selected, weighted and killed by cervical dislocation and necropsied. Liver, pancreas, testis and spleen were separated and weighted.

Statistical analysis

A completely randomised design with a 2 x 3 factorial arrangement of treatments was used to evaluate the response of broiler chickens to 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 for multiple treatment comparisons. For all tests, significance was declared at p < .5.

### Results

DWG and EPI were reduced by 0.48 g (6.67%) and 2.94 unit (13.62%) and FI and FCR were increased by 0.88 g (3.76%) and 0.39 (12%) in the birds fed on diets containing 2.2 mg/kg aflatoxin, respectively, (p < .05; Table 2). A significant aflatoxin x silymarin (A x S) effect observed in FI, DWG, FCR and PEI (p < .05), where FI increased in the birds maintained on the aflatoxin-contaminated diet and drinking water supplemented with free silymarin compared with all other birds. Moreover, birds having access to silymarin-nanohydrogel in drinking water exhibited greater DWG than all birds except those received the control diet (Table 2). Mean serum ALP activity in birds grown on the aflatoxin-contaminated diet (453.166 U/L) was greater (by 26.08%) than those fed with the control diet (p < .05; Table 3). Mean serum ALP and LDH activity were influenced by S x A effect (p < .05) so that activity of both enzymes elevated in birds grown on the aflatoxin-added diet and free silymarin-contained water compared with all other birds (Table 3). Mean serum Ca level decreased in the control birds compared with

### Table 2. Effects of different forms of silymarin in aflatoxin contaminated diets on productive performance of Japanese quail during days 7–35 of age.

| Aflatoxin (mg/kg) | Silymarin (mg/kg) | FI (g/day/bird) | DWG (g/day/bird) | FCR (g:g) | Mort. (%) | EPI |
|------------------|------------------|----------------|-----------------|-----------|-----------|-----|
| 0                | 23.39 b          | 7.20           | 3.25b           | 0.00      | 21.58 a   |     |
| 2.2              | 24.27 a          | 6.72b          | 3.64a           | 0.08      | 18.64b    |     |
| SEM              | 0.02             | 0.02           | 0.01            | 0.06      | 0.16      |     |
| 2.2              | 25.57 b          | 6.96b          | 3.70b           | 0.13      | 21.05a    |     |
| 500 free         | 23.49 b          | 3.79           | 3.25b           | 0.00      | 20.96c    |     |
| SEM              | 0.03             | 0.03           | 0.02            | 0.07      | 0.19      |     |
| 0                | 0                | 24.32 b        | 3.24            | 0.00      | 23.93 a   |     |
| 0                | 500 free         | 21.43 d        | 3.72b           | 0.00      | 20.71 b   |     |
| 0                | 500 nano         | 24.43 b        | 3.54           | 0.00      | 20.64 a   |     |
| 2.2              | 0                | 22.65 c        | 4.12           | 0.00      | 18.70 d   |     |
| 2.2              | 500 free         | 24.44 b        | 4.12           | 0.00      | 21.28 b   |     |
| SEM              | 0.04             | 0.04           | 0.03            | 0.10      | 0.27      |     |

*p Value

| Aflatoxin (A) |       |       |       |       |       |       |
|---------------|-------|-------|-------|-------|-------|-------|
| Silymarin (S) | <.0001| <.0001| <.0001| <.0001| <.0001| <.0001|
| S x A         | <.0001| <.0001| <.0001| <.0001| <.0001| <.0001|

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

Fl, feed intake (g/day/bird); DWG, daily weight gain (g/day/bird); FCR, feed conversion ratio (g:g); Mort., mortality (%); EPI, European production index. 500 free, silymarin free form (500 mg/kg drinking water); 500 nano, silymarin-nanohydrogel form (500 mg/kg drinking water); SEM, standard error of the mean (n = 12).

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those receiving either aflatoxin or silymarin as well as their combinations (Table 4).

Mean HDL in the birds that received aflatoxin-contaminated diet was 36.416 mg/dL (27.06%) which was lesser than those fed with the control diet ($p < .05$; Table 5). The birds that were given diets with 2.2 mg/kg aflatoxin had greater percentage of liver and spleen ($p < .05$; Table 6). Feed intake and DWG were greater (3.65% and 8.05%, respectively), while FCR was lesser (4.13%) by the birds provided by 500 mg/kg silymarin-nanohydrogel in 1 L of drinking water compared with the control birds on days 7–35 of age ($p < .05$; Table 2). The birds that received drinking water containing 500 mg/L silymarin-nanohydrogel had greater ALP activity (13.86%) ($p < .05$; Table 3). Mean serum concentration of BUN (0.785 mg/dL – 86.34%) and GLU

| Aflatoxin (mg/kg) | Silymarin (mg/kg) | ALT (U/L) | AST (U/L) | ALP (U/L) | GGT (U/L) | LDH (U/L) |
|------------------|------------------|-----------|-----------|-----------|-----------|-----------|
| 0                | 0                | 14.33     | 197.83    | 25379.1   | 2.64      | 645.41    |
| 2.2              | 17.16            | 15.84     | 180.26    | 93.2      | 655.00    |
|                  | 13.00            | 217.62    | 1802.62   | 3.92      | 655.00    |
|                  | 21.25            | 153.37    | 1973.87   | 2.92      | 823.75    |
|                  | 13.00            | 195.20    | 2073.00   | 2.95      | 564.87    |
|                  | 3.05             | 19.40     | 58.17     | 0.57      | 56.77     |
| 0                | 11.25            | 211.00    | 1782.75   | 2.76      | 653.50    |
| 2.2              | 18.50            | 179.66    | 2191.08   | 3.89      | 723.66    |
|                  | 14.75            | 224.25    | 1858.98   | 5.08      | 676.50    |
|                  | 24.00            | 132.75    | 2272.75   | 3.13      | 980.00    |
|                  | 12.75            | 182.00    | 2420.00   | 3.46      | 509.50    |
|                  | 4.32             | 27.43     | 82.27     | 0.81      | 80.29     |

| p Value          |                  |           |           |           |           |           |
|------------------|------------------|-----------|-----------|-----------|-----------|-----------|
| Aflatoxin (A)    | .0058            | .429      | <.0001    | .0764     | .2481     |           |
| Silymarin (S)    | .0494            | .0856     | .019      | .394      | .0156     |           |
| S × A            | .3104            | .1344     | .0224     | .5006     | .041      |           |

Alt, alanine amino transferase (U/L); AST, aspartate amino transferase (U/L); ALP, alkaline phosphatase (U/L); LDH, lactate dehydrogenase (U/L); GGT, gamma-glutamyl transaminase (U/L); 500 free, silymarin free form (500 mg/kg drinking water); 500 nano, silymarin-nanohydrogel form (500 mg/kg drinking water); SEM, standard error of the mean ($n = 4$).
Effects of different forms of silymarin in aflatoxin-contaminated diets on serum lipid profile concentrations of Japanese quail during days 7–35 of age.

| Table 5. Effects of different forms of silymarin in aflatoxin-contaminated diets on serum 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) |
| 0 | 178.00 | 31.66 | 134.58* | 99.08 | 0.21 | 0.04 | 0.16 |
| 2.2 | 150.25 | 31.25 | 98.16* | 87.16 | 0.19 | 0.04 | 0.14 |
| SEM | 11.12 | 8.06 | 4.18 | 5.95 | 0.00 | 0.00 | 0.00 |
| 0 | 174.25 | 51.12* | 111.00 | 108.00 | 0.18 | 0.05 | 0.13 |
| 500 free | 162.12 | 28.75* | 116.75 | 83.50 | 0.21 | 0.04 | 0.16 |
| 500 nano | 156.00 | 14.50* | 121.37 | 87.87 | 0.21 | 0.04 | 0.16 |
| SEM | 13.62 | 9.87 | 5.12 | 7.28 | 0.01 | 0.00 | 0.01 |
| 0 | 182.50 | 47.75 | 139.25 | 123.25 | 0.17 | 0.04 | 0.13 |
| 500 free | 186.50 | 39.25 | 130.00 | 87.00 | 0.23 | 0.05 | 0.18 |
| 500 nano | 165.00 | 8.00 | 134.50 | 87.00 | 0.23 | 0.05 | 0.18 |
| 0 | 166.00 | 54.50 | 82.75 | 92.75 | 0.18 | 0.05 | 0.12 |
| 2.2 | 137.75 | 18.25 | 103.50 | 80.00 | 0.20 | 0.04 | 0.16 |
| 0 | 147.00 | 21.00 | 108.25 | 88.75 | 0.18 | 0.03 | 0.15 |
| 2.2 | 12.49 a | 1.20 0.26 0.09 b | 2.94 b | 1.50 0.23 0.09 b | 0.10 | 0.15 | 0.01 | 0.00 |
| SEM | 19.27 | 13.96 | 7.24 | 10.30 | 0.01 | 0.00 | 0.01 |

*Means within the same column with different letters are significantly different by Tukey’s test (< 0.05).

Discussion

Huge number of reports have been appeared in the literature concerning poultry demonstrating adverse effects of aflatoxin on the performance and health in broiler chicken (Yunus et al. 2011), turkey (Rauber et al. 2007), ducks (Chang et al. 2016) and quail (Oliveira et al. 2002; Bagherzadeh Kasmani and Mehri 2015; Khaleghipour et al. 2019). In a study, feeding quail chicks with aflatoxins-contaminated diets (0.25, 0.5, 1 and 2 mg/kg feed) significantly reduced FI, DWG and increased FCR (Mahmood et al. 2017). Sakamoto et al. (2018) reported reduced FI (1.59 g or 5.75%) and impaired FCR (0.05 unit or 1.3%) in quails fed with aflatoxin-contaminated diets (1500 μg/kg) compared with the unchallenged birds. Results of the current study is in the line with almost all previous reports that confirmed decreased DWG and EPI and increased FCR in quails fed aflatoxin-contaminated diets. In the contrary to previous studies, feeding quail chickens with an aflatoxin-contaminated diets increased FI. However, in agreement with our finding the increased FI (27.8%) was reported by Manafi (2018) in quails fed aflatoxin-contaminated diets (1500 μg/kg) compared
with the unchallenged ones. The adverse effects of aflatoxin in birds are mainly attributed to liver dysfunction manifested by anorexia, listlessness, inhibition of protein synthesis and lipogenesis in the contaminated birds (Basmacoglu et al. 2011). Aflatoxin B1 also imposes an adverse effect on the absorption of macro nutrients and reduces the activity of the digestive enzymes (Manafi et al. 2014). In the current study, the decreased DWG in aflatoxin-challenged birds can be attributed to several impaired physiologic processes. At the outset, aflatoxins cause enterocytes necrosis and atrophy leading to reduced villi surface area and apparent absorptive surface area in gastrointestinal tract, a phenomenon which is responsible for reduced nutrient absorption (Awad et al. 2008; Manafi 2018). Secondly, aflatoxins are potent toxins (Rotimi et al. 2017) which impair functionality of enzymes and substrates involving in protein synthesis, cell proliferation and gene expression in many tissues, in particular, liver (Shuaib et al. 2010). In the course of aflatoxicosis, proportional weight of liver, kidney and other organs directly involved in the detoxification may increase to clean the body from harmful substances, as indicated in the current study by enhanced liver and spleen weight, the results which agree Ledoux et al. (1999) and Şehu et al. (2005) findings. Increased metabolism coincides with impaired biological membranes in liver cells in aflatoxin-treated birds could result in infiltration of certain intracellular substrates and entities. In this study, ALP activity elevated in quails fed on the aflatoxin-contaminated diets, which is considered as an indication of liver injury (Gowda et al. 2008). While liver plays a central role in aflatoxin detoxification, altered serum levels are anticipated for many biochemical components as observed in the current study. Finally, aflatoxins such as almost all other toxicants induce anorexia (Şehu et al. 2005; Yunus et al. 2011) through influencing central appetite modulating mechanisms (Sakamoto et al. 2018), resulting in a lesser DWG.

During the past few decades, many mycotoxin absorbents in different physical, chemical and biological categories have been introduced and adopted widely in poultry industry (Ismail et al. 2018) to guard the birds against the aflatoxins present in diet. Recent focus on herbal remedies has created a new hope for safe, effective and inexpensive mycotoxin counteracting feed additives. Milk thistle (S. marianum) extract known as silymarin, among many others, has received huge attention in poultry researches. In the present study, the significant aflatoxin × silymarin effects exhibited as increased FI and DWG and decreased FCR, LDL, GLU and Ca were interesting in the birds that received drinking water containing silymarin-nanohydrogle. Moreover, birds with access to the nanohydrogle-added water exhibited greater DWG provided further indication for possible mycotoxin-counteracting effects of silymarin. These results are in the line with the findings of Neshat Gharamaleki and Mohajeri (2015).

Calcium is a vital electrolyte in normal cell function of many tissues hence its hemostasis regulated by feedback mechanisms controlled by several hormones in a narrow range. We speculated that the modified serum Ca concentration in the birds provided with silymarin in either free- or nanohydrogle forms may be due to potential silymarin binding with the free serum ionised Ca.

Moreover, the proportional weights of liver and spleen decreased in the birds that fed on the aflatoxin-contaminated diet and received silymarin-nanohydrogle water giving further indication for effectiveness of this novel approach. We speculate that silymarin-nanohydrogle increases the bioavailability of the silymarin and enhances its intestinal low absorbing rate. As a result, greater levels of silymarin active ingredients in blood stream may induce insulin secretion by beta cell of the pancreas (Soto et al. 2004), promote renovation of the pancreatic tissue, protect pancreatic tissue against damaging metabolites, thereby exert hypoglycaemic effect among many other mechanisms pointed out as silymarin beneficial effects on quail performance and health (Soto et al. 2003; Behboodi et al. 2017).

**Conclusions**

Feeding diets contaminated with 2.2 mg aflatoxins/kg impaired growth performance and adversely altered hepatic function in Japanese quails. Adding 500 mg/kg silymarin-nanohydrogle in drinking water of the same birds alleviated the adverse impacts of aflatoxins on bird’s performance and in partial, liver function. Greater levels and/or another administration forms of silymarin-nanohydrogle may relieve the aflatoxin-caused intimidating alterations in liver and blood parameters studied, a suggestion which warrant further research in the same field.

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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.

Health and safety
Authors confirm that all mandatory laboratory health and safety procedures have been complied with in the course of conducting all experimental works reported in this paper.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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