Efficacy of Silymarin on Aflatoxin Residues in Eggs and Health Status of Laying Hens

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Abstract

The study aimed to evaluate the silymarin in reducing the aflatoxin (AF) residues in eggs and health status of laying hens. A total of 120 laying hensat the age of 240 days were randomly divided into three equal groups fed on diet with or without AF and 0.5% of silymarin. The residues of AF in the eggs were measured at the age of 260 and 280 days by using HPLC technique as well as, physiological tests. The results of the 3rd group showed a significant (P≤0.05) increase in RBCs, PCV% and Hb, AST, ALT concentrations and Ab titer against ND and IB diseases as compared with the 2nd group and the control. Also, there was a significant (P≤0.05) decrease in the concentrations of AF (B1) and (G1) in eggs at the age of 260 days as compared with the 2nd and the control.

Keywords: *Aflatoxin residue, Silymarin, HPLC technique.*

Introduction

The main problem in poultry industry represented via contamination with different mycotoxin(1). Aflatoxin (AF) contaminated grains are a worldwide problem that leads to immunosuppression and an altered response to vaccination programs in poultry. These effects result in a decline in animal performance and ultimately minor profitability(2).High levels of AF in feed results decrease in production performance, and the prevalence of residues in poultry meat and eggs(3). The toxicity of AF mainly depends on the quantity and duration of ingestion and immune status, as well as environmental factors(4).

Silymarin (SM) an extractedas of the kernels of milk thistle (*Silybummarianum*), is acomposite of flavonolignans that has activityof free radical scavenging. Silymarin is affect positively on the digestibility in the birds that enhance the feed efficiency(5). Milk thistle seed has been identified as protective nutrient of poultry from adverse effects of AFB1(6,7) described that the supplementation of SM by 0.5% of feed had a positive effect to reduce negative effect of mycotoxins with improved of the performance and health status for broilers. Muhammad(8) reported that SM enhanced liver cell protein synthesis also inhibited oxidation of glutathione. The goal of the present study is evaluate the Silymarin to reduce the AFresidue in eggs and health status of laying hens.

Materials and Method

A total of 120 hens (ISA Brown) were randomly divided into three equal groups at 40 hensaged 240 days old were obtained from commercial farm in Diyala province. All birds were offered feed adjusted at a rate of 4400 gm/day/group(9) (table 1) with water (*ad libitum*). The 1st group (control) was fed a diet free of mycotoxin. The 2nd group fed contaminated diet byaflatoxin (14.6 ppb) while the 3rd group fed the same diet with 0.5% of silymarin. At aged 242 the birds were vaccinated with attenuated IB(4/91) +ND Clone 30 viadrinking water.

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Table 1. The compositions of the diets were prepared for this study

Ingredients	Weight (kg)
Yellowcorn	47.0
Soybean meal (45% crude protein)	22.0
Wheat by product	20.0
*Animal feed premix 2.5%	2.5
Sunflower oil	0.5
Limestone	6.5
**Dicalcium phosphate	1.0
Salt	0.5
Total weight (kg)	100
Calculated chemical analysis	
Crude protein %	16.60%
Metabolizable Energy (kcal/kg)	2711.21
Calcium (%)	2.718
Available phosphorus (%)	0.750
Methionine (%)	0.297
Lysine (%)	1.725

*Animal feed premix for hens(Laymix-2.5 W) Holland made. Inclusion rate: 2.50%. Chemical contains: Crude Protein 11%, Crude Fat 2.18%, and Crude Fiber 0.84.**Dicalcium phosphate Iraq made contains: Ca 23% and P 18%.

Blood samples were taken from each group for physiological tests[10,11] at aged 280 days, as well as, the residues of AF in the eggs were measured at the age of 260 and 280 days by using high-performance liquid chromatography (HPLC) technique. The egg samples collected randomly to test in the laboratories of ministry of science and technology. Data was analyzed with SAS software[12]using LSD and ANOVA to compare between treatments.

Results and Discussion

The results showed significant (P≤0.05) increased in RBCs of the 3rd group as compared with the 2nd and control (Table 1) whiles the count of WBCs recorded lower values in 2nd group than other groups. The increasing of RBCs count may be Silymarin (SM) 's ability to keep blood cells from any damage caused by free radicals in the body that may be damaging to cell membranes and their internal components in the body by acting as an antioxidant. Silymarin has anti-oxidant ability as it works directly or indirectly for cracking and elimination of many compounds such as phenylglyoxylic also radical'sketyl[13].Also, that explanation was similar with reported by[14].

Table 1. Effect of Silymarin on blood picture of different groups at age 280 days (Mean ± SE).

Groups Parameter	Group 1	Group 2	Group 3
WBC(Cell×103/ml)	83.47 ± 2.45B	83.12 ± 5.17B	90.1 ± 1.72A
RBC(Cell×106/ml)	1.7 ± 0.06B	1.28 ± 0.19B	2.8 ± 0.31A
Hb(gm/dl)	8.77 + 0.65B	6.82 + 1.03C	13.27 + 1.64A
PCV%	24.57 + 1.52B	18.17 + 2.66C	36.88 + 4.68A

Means in the same row with different letters differ significantly (P≤0.05).

Nazifi and Asasi[15] concluded that Silymarin supplementation at percentage (0.5 and 1%) did not alteration the values of the studied hematological parameters (WBC,RBC) as compared to the control group.[16] found that SM significantly helps to keep the blood cells and serum biochemical parameters in normal range. The results of total protein did not record significant differences (P≥0.01) among groups at 40 days of study (Table 2). While, the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) dataenzymes activity of the 2nd group was recorded significantly (P≤0.01) higher values as compared with those in the 3rd and control groups. The increased AST and ALT activities detectedvia feeding polluted diets in the present study may be the effects of AF which cause hepatic degeneration and subsequent leakage of enzymes into circulation. The increasing in liver enzymes activity may be the most sensitive indicator of liver damage[17],[18] said that any liver enzymes disorder is evidence of stress. The results agreed with[19] who referred to the

AF has hepatotoxic effects and the activity of AST and ALT might be increased. While the reduction in the liver enzymes activity may be due to the positive effect of SM in protect the hepatic cells from damage by AF. The antioxidant property of Silymarin against free radicals is what prevented hepatocytes from being damaged[20]. Silymarin as well promotes hepatocytes protein creation also declines the oxidation of glutathione [8].

Table 2. Effect of Silymarin in total protein and liver enzymes for different groups at age 280 days (Mean ± SE).

Groups Parameter	Group 1	Group 2	Group 3
AST (U/L)	90.0 ± 17.3B	145.0 ± 30.0A	107.5 ± 37.7B
ALT (U/L)	50.6 ± 34.9B	145.0 ± 28.8A	102.5 ± 55.6B
Total Protein (g/L)	6.93 ± 0.9A	7.23 ± 1.1A	7.29 ± 1.2A

Means in the same row with different letters differ significantly (P≤0.01).

Anti-oxidative mediators are crucial for hens nutrition as they declinefat peroxidation, consequently increase the organoleptic features,in addition nutritional value of meatthenegg, increased meat and egg shelf life[21].The most well-known exogenous antioxidant substanceslike vitamin E[22], Oligo-elements like, selenium,amino acids, glycine also flavonoids such as SMneed been identified[23].

By using ELISA test, the results of the mean titers of anti-ND and IB vaccines are non- significant (P>0.05) differences among groups at 20 days of study, but there was significant (P≤0.05) increase in Ab titers in the 3rd group as compared with the 1st and 2nd groups at 40 days of the beginning of the study (table 3). The increasing in Ab titer against ND vaccine may be attributed to the amelioration effect after feeding onration treated with the Silymarin which preserves the liver cells from aflatoxin damage, that action enhancedprotein synthesis in the body which promotion the immune response of chickens. Humoral immune response was development after added of silymarin is in line with[8,20]. Moreover, the effect of hydrated sodium calcium aluminosilicate on the humeral immune response of quails diet on AFB1 polluted ratio was studied by [24]also found decrease in the Ab titer induced by ND vaccine, due to AF, was relatively prevented. However, the lowering in Ab titer of the 2nd group attribute to contaminated diet with AF.[25] take down resistance to diseases revealed that AF also inhibits with vaccine-inducing immunity in animals. Manegar[26] established that AF destroy prime immune response aimed at ND also Gumboro disease as apparent via drop in the ELISA titers. Table (3) shows the Ab titers of 2nd group were significantly (P≤0.05) the lowers than the 3rd group. It is clear from our observations and those of other researchers that AF causes severe immunosuppression that might be due the depressed the phagocytic activity[27]. The results are supported through[28] who informed the negative role of contaminated feed in the presence of immune suppressant AF in diet. Whilst feed addition of SM with AF contaminated ration in 3rd groups significantly (P≤0.05) improved the immune response of hens.

Table 3: Effect of Silymarin in Immunity against ND and IB for different groups at different age (Mean ± SE)

Type of vaccine	Groups Parameter	Group 1	Group 2	Group 3
ND	240 Days	21930.16 ±2376.83	21930.16 ±2376.83	21930.16 ±2376.83
	260 Days	24686.25 ± 1250.48	27877.50 ± 1757.52	24968.00 ± 3844.96
	280 Days	13723.00± 482.00B	13205.83 ± 588.23B	17696.55 ± 778.41A
IB	240 Days	46388.50 ± 4711.85	46388.50 ± 4711.85	46388.50 ± 4711.85
	260 Days	31506.50 ± 418.99	33756.80 ± 2614.56	33582.00 ± 336.99
	280 Days	52614.00 ±2511.66A	40944.33 ± 1979.30 B	47792.50± 3141.21 A

Means in the same row with different letters differ significantly (P≤0.05).

Moreover, Silymarin supplementation with AF contaminated diet can reduce the adverse effects of AF on performance and systemic immune response. These findings suggest that SM play as an antioxidant property via to prevent the negative effect on immune system. The researchers Friedman[29] confirmed that estimation Ab titers in birds' serum give good evidence to appointment immunity of birds against ND virus. Thus, the positive results indicated that the effect of SM in preservation of the immune cells and keep cellular membranes flexibility which have achieve a role in antigen diagnosis[30] .

In this study, the concentrations of AF B1 and G1 were evaluated by HPLC, figure 1 illustrates the standard chromatogram of the AF B1 and G1 at retention times from 4.5 to 5 minute.

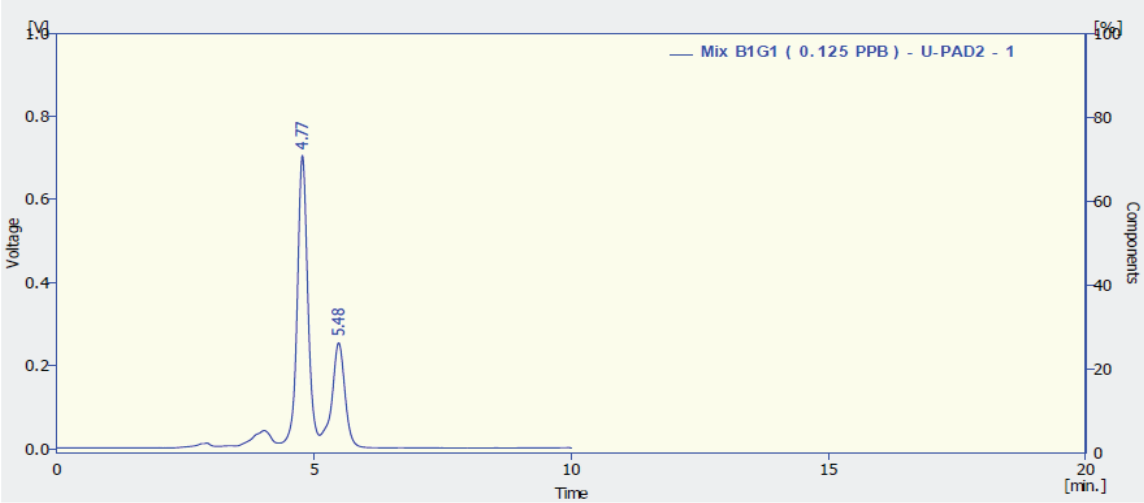

Figure 1. Standard chromatogram of the AF B1 and G1

The results obtained from the analysis the eggs samples of the 2nd group during 20 days of the beginning of the study are represented in figure 2. The predominant AF for all analyzed samples was mixed B1and G1. The test indicated that the transmission is relatively persistent during the processing of eggs formation. Trucksess[31], the residues of AF didn't seem eggs until the day 4 of feeding polluted diet. Dissimilar recorded by[32], AF residues seem in the eggs when hens feeding with 5 mg/kg AF only also/or in mixture with Ochratoxin at 3 and 5 mg/kg. Also,[33] watched AFB1 also the aflatoxin, in the layer hens eggs after feeding the diet polluted with AF. used immune-affinity column with HPLC.

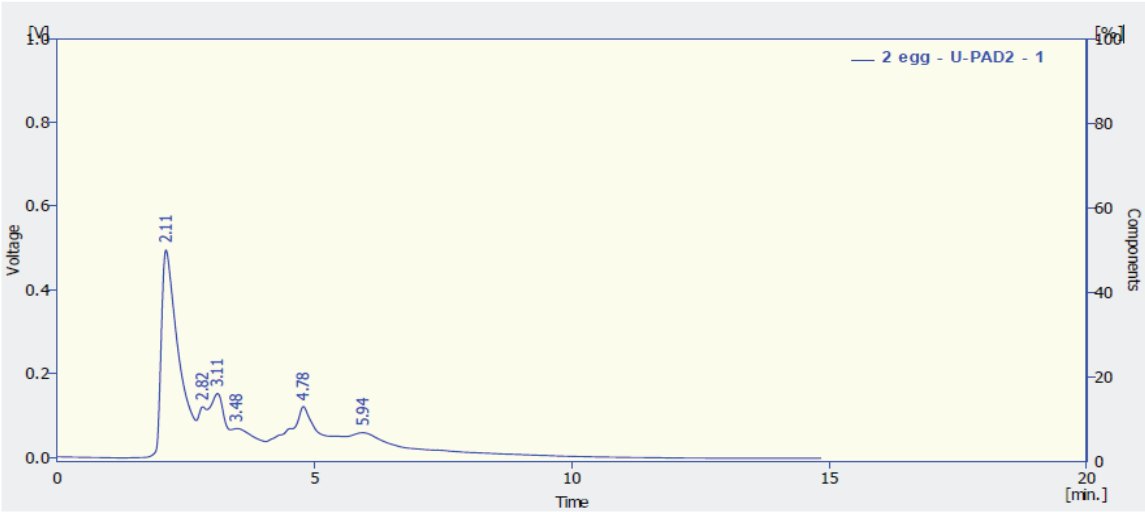

Figure 2: Chromatogram of total AF B1 and G1 residues in eggs of the 2nd group

Hussain[34] is found direct link between AFB1 in the diet and residues in the eggs and muscles. AFB1 could contaminate the food manufactured from laying hens or eggs raised on AF polluted feed. According to the present study, it can conclude that silymarin reducing the aflatoxin residue in eggs and improving production efficiency and enhance health status of laying hens. So, the use of Silymarin at the level of 0.5% in feed during the production cycle seems to offer the best benefits.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

Conflict of Interest: Non

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