



## Ameliorating effects of silymarin against mycotoxin and its effect on some production and hematological parameters of broilers

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Received: October 16<sup>th</sup> 2020; Accepted: November 14<sup>th</sup> 2020

### Abstract

This study aims to evaluate the efficacy of silymarin (*Silybum Marianum*) on some productions and Hematological parameters of broilers. A total of 160 chicks (Ross-308) at one day old were used in this study, randomly distributed to four equal groups (40 chicks / group) with two replicates of each replicate. The first group was fed on a basal diet. The second group fed on contaminated diet with mycotoxins. The third group fed on basal diet with 0.5% Silymarin/ kg feed. The fourth group fed on contaminated diet with mycotoxins + 0.5% Silymarin/ kg feed. Production traits were calculated weekly, and blood samples were collected at 35 days for haematological tests. The results showed superior significant differences ( $P \leq 0.01$ ) in live body weight, weekly weight gain and improvement in feed consumption, and feeds conversion ratio of the 3<sup>rd</sup> group as compared with the other groups as well as significant increased ( $P \leq 0.01$ ) in WBC, RBC and PCV% compared with the 2<sup>nd</sup> and 4<sup>th</sup> groups. In conclusion, the supplementation of Silymarin (*Silybum Marianum*) by 0.5% / kg feed had minimized the adverse effects of toxins contaminated feed on the performance of broilers.

**Keywords:** Mycotoxins, Productions traits, Silymarin, Milk thistle, Haematological tests.

Research paper from MSc thesis for the second author.

### Introduction

Mycotoxins are biologically active, toxic metabolites formed by secondary metabolism of fungal genera, such as *Aspergillus*, *Fusarium* and *Penicillium* species, which attack crops in the field and may grow on foods during packing under favorable conditions of temperature and humidity (Shamsudeen *et al.*, 2013). Mycotoxin contaminated grains are a worldwide problem that leads to immunosuppression and an altered response to vaccination programs in poultry, as well as decline in animal performance and ultimately minor profitability (Binder, 2007, Anjum *et al.*, 2011). Exposure to mycotoxins occurs mostly by the ingestion of contaminated cereals such as corn, wheat, peanuts and sorghum, in addition to other raw materials used in the preparation of animal feed (Sobrane Filho, *et al.*, 2016). High levels of mycotoxins in feed results the appearance of acute mycotoxicoses and high mortality rate while lower levels cause the prevalence of chronic mycotoxicoses with or without showed clinical symptoms, but followed by a considerable decrease in production performance, immunosuppressive effects, and the prevalence of residues in poultry meat and eggs (Cheng *et al.*, 2000). Silymarin an extract from the seeds of milk thistle (*Silybum marianum*), is a complex of

flavonolignans that have robust antioxidant and free radical scavenging activity. It has been exposed that milk thistle seed (MTS) defends birds against adverse effects of Aflatoxin B1 (AFB1) (Federico *et al.*, 2017). Milk thistle extracts have been used to treat diseases ( Schrieber *et al.*, 2008). Silymarin are also used to successfully as hepatoprotective, anti-inflammatory, cytoprotective, and anticarcinogenic effects (Stiuso *et al.*, 2014). Its mechanism of action still seems to be poorly understood, but data in the literature suggest that it acts as an antioxidant, cell membrane stabilizer and permeability regulator, in addition to promoter of DNA, RNA, and protein synthesis (Vargas-Mendoza *et al.* 2014).

### Materials and Methods

One hundred sixty broiler chicks strain (Ross 308) at one day old were used in the current study. The experiment conducted in the poultry farm of the Veterinary Medicine collage/ University of Baghdad for 35 days from 18 January to 21 February 2019. The first group (control) was fed with basal diet which determine according to NRC (1994) as in (table 1).

The starter diet was applied for 21 days of old, then replace with the grower diet up to end of the experiment. The second group: chicks were Fed on contaminated diet (feed contamination with

mycotoxins it was obtained from private factories, feed which contains yellow local corn and examined on the Al Nahda Laboratories / Veterinary Directorate and recorded this results (aflatoxins 8.4 ppb , T-2 toxin 24.3 ppb). The third group: chicks were Fed on basal diet with 0.5% Silymarin. The fourth group: chicks were fed on contaminated diet with mycotoxins and 0.5% Silymarin.

Blood samples were collected randomly (10 chicks from each group) for the heamatological tests with anticoagulant which was Potassium Ethylene

Diamine Tetra Acetic Acid (K3-EDTA) to measure WBC count, RBC count packed cells volume (PVC%) and haemoglobin concentration (Hb). these tests were conducted in the haematology unit/ laboratories department of Veterinary Directorate. The Statistical computations were done using SAS software program (SAS, 2012) to explore the influence of treatment. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study.

**Table (1) Composition of the experiment diets (Starter and grower) prepared in this study for all groups.**

Ingredients	Starter	Grower
Yellow corn	45.0	48.0
Soybean meal (45% crude protein)	26.0	22.0
Wheat	15.0	18.0
*Protein concentration (40% protein)	10.0	8.0
Sunflower oil	2.0	2.0
Lime stone	1.0	1.0
**Dicalcium phosphate	0.70	0.70
Salt ( NaCl )	0.30	0.30
Total weight (kg)	100	100
<b>Calculated chemical analysis</b>		
Crude protein %	21.70	19.90
Metabolizable Energy (kcal / kg)	2982	3026
Calcium (%)	1.34	1.19
Available phosphorus (%)	0.79	0.72
Methionine (%)	0.45	0.40
Lysine (%)	1.20	1.05

\* Protein concentration type (Provemi) Jordan made. Chemical contains: Protein 40% , M.E 2250 Kcal /Kg, Fat 6.5%, Fiber 3.5%, Ash 31.0%, Calcium7.0%, Available phosphorus 3.3%, Methionine 1.80%, Lysine 3.0%, Meth.+Cystine2.3%,Sodium 1.3%,Chloride 2.3%.

\*\* Dicalcium phosphate Iraq made contains: Calcium 23% and phosphorus 18%.

## Results and Discussion

### Growth performance:

**Live Body weight (g):** The results of live body weight were showed significant differences among treatments and control group at level ( $P \leq 0.01$ ) as shown in table (1). The third group was recorded the highest mean body weight in the (1<sup>st</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup>) weeks (146.17±2.01 g, 1015.00±19.79 g, 1815.00±42.03 g, 2702.50±50.75 g) respectively as compared with other groups at the same time. while there was no significant ( $P > 0.01$ ) differences among treatments and control group in first day of age. In addition, the results showed significant ( $P \leq 0.01$ ) increased in live body weight of the fourth group in (1<sup>st</sup>, 2<sup>nd</sup> and 5<sup>th</sup>) weeks (137.98±1.81 g, 397.50 ± 5.83 g, 2475.00±20.74 g) respectively as compared with the second group (125.61±1.77 g, 367.50 ± 8.37 g, 2315.00±20.13 g) respectively.

**Weekly weight gain (g)** The mean weekly weight gain was increased significantly ( $P \leq 0.01$ ) in the third group at the 1<sup>st</sup> and 3<sup>rd</sup> weeks as compared with the other groups are illustrated in Table (2). However, the third group showed significantly ( $P \leq 0.01$ ) higher weight gain than other groups and control during the period of this study, as well as, the fourth group reported significantly ( $P \leq 0.01$ ) higher value (2432.60± 20.73g) than those of the second group (2271.09±20.11 g) in total compared.

**Feed Intake (FI):** Feed intake was not affected during the 2<sup>nd</sup> and 3<sup>rd</sup> weeks of age in different groups. The third group recorded a significant ( $P \leq 0.01$ ) increase in feed intake as compared with the other groups in total compared with other groups table (3).

**Table (1) Effect of Silymarin on live body weight (g /bird) for different groups (Mean ± SE). Means in the same row with different letters differ significantly and \*\* indicates(P≤0.01).**

Group	Group 1 Control (Basal diet)	Group 2 (contaminated diet with mycotoxins)	Group 3 0.5% Silymarin	Group 4 0.5% Silymarin + mycotoxins	LSD values
Age					
1 <sup>st</sup> day	44.66± 0.93a	43.90±0.23a	44.82±0.48a	42.39±0.42a	1.66NS
1 <sup>st</sup> wk	137.86 ± 1.91 b	125.61±1.77c	146.17±2.01a	137.98±1.81b	5.399**
2 <sup>nd</sup> wk	422.50 ± 8.70 a	367.50 ± 8.37c	430.00± 7.26a	397.50 ± 5.83 b	21.87**
3 <sup>rd</sup> wk	890.00± 22.42 b	817.50 ± 19.02 c	1015.00±19.79 a	855.00 ± 9.71 bc	52.70**
4 <sup>th</sup> wk	1682.50±24.73 b	1485.00±15.00 c	1815.00±42.03 a	1540.00±17.15 c	77.20**
5 <sup>th</sup> wk	2480.00±46.96 b	2315.00±20.13 c	2702.50±50.75 a	2475.00±20.74 b	107.48**

**Table (2) Effect of Silymarin on weekly weight gain (g/bird) for different groups (Mean ± SE).**

Group	Group 1 Control (Basal diet)	Group 2 contaminated diet with mycotoxins	Group 3 0.5% Silymarin	Group 4 0.5% Silymarin + mycotoxins	LSD values
Age					
1 <sup>st</sup> wk	93.20±1.85b	81.70 ± 1.63c	101.35 ±2.40a	95.64 ±1.78b	5.57**
2 <sup>nd</sup> wk	284.62±9.66a	241.87 ± 8.49b	283.82 ± 7.22a	259.45 ±5.37b	22.52**
3 <sup>rd</sup> wk	467.50±26.36 b	450.00 ± 24.15 b	585.00±17.95 a	457.50 ±11.21 b	59.58**
4 <sup>th</sup> wk	792.50±22.68 a	667.50 ± 18.65 b	800.00±55.52 a	685.00± 21.47 b	95.20**
5 <sup>th</sup> wk	797.50±45.86 b	830.00 ± 22.91 ab	887.50±55.93ab	935.00± 18.71 a	112.07*
Total	2435.33±46.97 b	2271.09±20.11 c	2657.67±5.49a	2432.60±20.73 b	107.23**

Means in the same row with different letters differ significantly; \* indicates (P≤0.05), and \*\* indicates (P≤0.01).

**Table (3) Effect of Silymarin on feed intake (g feed/bird) for different groups (Mean ± SE).**

Group	Group 1 Control (Basal diet)	Group 2 contaminated diet with mycotoxins	Group 3 0.5% Silymarin	Group 4 0.5% Silymarin + mycotoxins	LSD values
Age					
1 <sup>st</sup> wk	137.50±12.150 ab	120.00 ± 10.00 b	162.50±12.50 a	123.75± 1.25 ab	39.94 *
2 <sup>nd</sup> wk	402.16±6.92a	432.64±209.14 a	419.44±19.44 a	411.13±6.37 a	57.98NS
3 <sup>rd</sup> wk	646.96±19.69 a	686.11 ± 36.11 a	722.07±16.81 a	674.54±10.36 a	89.57NS
4 <sup>th</sup> wk	1039.83±12.55 b	1068.06 ± 18.0 ab	1112.35±7.09a	976.54±11.54 c	50.71**
5 <sup>th</sup> wk	1334.71± 5.76 ab	1341.18 ± 41.17 ab	1442.32±31.20 a	1289.65±47.15 b	137.78*
Total	3561.16±7.06 bc	3647.98±125.48 b	3858.68±87.05 a	3475.72±53.58 c	318.05*

Means in the same row with different letters differ significantly; \* indicates (P≤0.05), and \*\* indicates (P≤0.01).

**Feed Conversion Ratio (FCR):** The data of table (4) demonstrated that the fourth group recorded significant (P≤0.01), which improved in feed conversion ratio in the 5<sup>th</sup> week as compared with the other groups. While there are no significant

differences (P>0.01) among different groups in the 1<sup>st</sup> week.

**Hematological parameters:** The haematological parameters exhibited significant (P≤0.01) differences among treatments and control groups at 35 days of age table (5).

**Table (4) Effect of Silymarin on weekly feed conversion ratio for different groups (Mean ± SE).**

Group	Group 1 Control (Basal diet)	Group 2 contaminated diet with mycotoxins	Group 3 0.5% Silymarin	Group 4 0.5% Silymarin + mycotoxins	LSD values
Age					
1 <sup>st</sup> wk	1.48 ± 0.14 a	1.46 ± 0.11 a	1.59 ± 0.10 a	1.29 ± 0.01a	NS
2 <sup>nd</sup> wk	1.41 ± 0.01 c	1.78 ± 0.04 a	1.47 ± 0.05 bc	1.58 ± 0.01 b	0.135**
3 <sup>rd</sup> wk	1.37 ± 0.01 ab	1.52 ± 0.06 a	1.23 ± 0.09 b	1.47 ± 0.00 a	0.221**
4 <sup>th</sup> wk	1.30 ± 0.03 c	1.59 ± 0.03 a	1.38 ± 0.01 bc	1.42 ± 0.00 b	0.097**
5 <sup>th</sup> wk	1.67 ± 0.06 a	1.61 ± 0.01a	1.62 ± 0.01a	1.37 ± 0.01 b	0.121**
Total	1.44 ± 0.06 b	1.59 ± 0.06 a	1.46 ± 0.02b	1.43 ± 0.05 b	0.123 *

Means in the same row with different letters differ significantly; \* indicates (P≤0.05), and \*\* indicates (P≤0.01).

**Table (5) Effect of Silymarin on blood pictures in different groups (Mean ± SE).**

Group	Group 1 Control (Basal diet)	Group 2 contaminated diet with mycotoxins	Group 3 0.5% Silymarin	Group 4 0.5% Silymarin + mycotoxins	LSD values
Parameter					
WBC x 10 <sup>3</sup> /ml	91.63 ± 0.41 a	88.06 ± 0.53 b	92.96 ± 0.13 a	89.00±0.57 b	1.464**
RBC x 10 <sup>6</sup> /ml	2.11 ± 0.05 a	1.56 ± 0.03b	2.22 ± 0.05 a	1.68 ± 0.01b	0.141**
PCV%	30.80 ± 0.45 a	23.20 ± 0.36 b	31.00 ± 0.30 a	23.56±0.46 b	1.315**
Hb g/dl\	9.50 ± 0.15 a	7.63 ± 0.12 c	9.70 ± 0.11a	8.70 ± 0.06 b	0.380**

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

The results showed that (WBC), (RBC), (PCV%) and (Hb) significantly (P≤0.01) decreased in second and fourth groups at 35 days of age, whereas (88.06x10<sup>3</sup> /ml ± 0.53, 89.00x10<sup>3</sup> /ml ± 0.57) (1.56x 10<sup>6</sup> /ml ± 0.03, 1.68x 10<sup>6</sup> /ml ± 0.01) (23.20% ± 0.36, 23.56% ± 0.46) (7.63 g/dl ± 0.12, 8.70 g/dl ± 0.06) respectively as compared with first and third groups whereas (91.63x10<sup>3</sup> /ml ± 0.41, 92.96x10<sup>3</sup> /ml ± 0.13) (2.11x10<sup>6</sup> /ml ± 0.05, 2.22x10<sup>6</sup> /ml ± 0.05) (30.80% ± 0.45, 31.00% ± 0.30) (9.50 g/dl ± 0.15, 9.70 g/dl ± 0.11) respectively. However, the fourth group established significantly (P≤0.01) higher value (8.70 g/dl ± 0.06) in Hb concentration than the second group (7.63 g/dl ± 0.12).

**Growth performance:** The results of the current study were showed significant differences in live body weight of experiment chicks at level (P≤0.01) among treatments and control group at the different periods as showing in the table (1).

The results of the present study demonstrated that silymarin had a significant role on growth because the improvement in absorption availability of nutrients accompany with the positive effects of silymarin as an antioxidant element which protect nutrient from oxidation, so the most feed in commercial diets especially the essential ingredients became available to the birds to profit from all it. These results in the present study agreed with Kalorey *et al.*, (2005) and Surai (2015) whom reported that silymarin improved body

weight in the presence of mycotoxins in feed. The increase in body weight of the current experiment birds might be due to the action of silymarin which decreased the toxicity of AFB1 and showed a positive effect on body weight in the broilers (Fani *et al.*, 2013). Zahid and Durrani (2007) revealed that the breast weights were significantly better when broilers fed silymarin supplementation at levels of 15 g/ kg diet as compared with control. Various trials showed that silymarin addition in diet or silymarin administration increased productive and reproductive performances and improved livestock health status of animals (Zarei *et al.*, 2016). Muhammad *et al.*, (2012) found improved body weight gain in the broiler fed with 10g/kg of milk thistle supplementation. in the same line that improved weight gain in milk thistle treated group as compared to birds raised on aflatoxin-contaminated feed alone (Tedesco *et al.*, 2004). Similarly, Abdalla *et al.*, (2018) reported the highest of body weight in the group supplied with 25 g/kg of silymarin. Asghar and Masood (2008) demonstrated that silymarin may be used in preventing free radical-related diseases as a dietary natural antioxidant supplement like the most vitamins especially vitamin E (Abdulwahid, 2015). It is suggested that silymarin can enter into the nucleus and act on RNA polymerase enzymes and the transcription of rRNA, resulting in increased ribosomal formation. This, in turn,

hastens protein and DNA synthesis (Sonnenbichler, 1986), which enhances the biosynthetic apparatus in the cytoplasm, thus leading to an increase in the synthesis rate of both structural and functional proteins.

The results of the current study were showed significant differences in means weekly weight gain of experiment chicks at level ( $P \leq 0.01$ ) among treatments and control group at the different periods as showing in Table (2). Higher weight gain was also reported by (Gowda and Sastry, 2000), in milk thistle supplemented groups. Silymarin promotes digestibility and absorption of protein in animal's body. The gradual positive effect of milk thistle in the broiler diet enhanced the digestibility and absorption of nutrients by increasing the activity of digestive enzymes (Sultan et al., 2018). This study indicated that silymarin may be reduced the toxic effect of mycotoxins in the intestine, also silymarin can act as a chelating agent, and in this way it could also interfere in the formation of toxins and in microbial growth and provide a beneficial absorption of nutrients (Hao, 2015, Radhika et al., 2016). Also, Fani et al. (2013) suggest that treatment with silymarin may be effective in counteracting the negative effects of AFB1 intoxication on feed intake and BWG in growing broilers. The results of FI showed significant ( $P \leq 0.01$ ) increase of the study chicks at the total compared in the third group as compared with other groups as showing in table (3). Silymarin supplemented groups had higher FI as compared to control. It may be due to its positive effects on metabolism and digestion. On the other hand, the presence of silymarin in mycotoxins contaminated feed improved feed intake. These results were in agreement with (Kalorey et al., 2005). The present results agreed with (Tedesco et al., 2004), who observed improved FI in silymarin treated group as compared with the birds fed on AFB1 contaminated feeds only.

Similarly, Muhammad et al., (2012) confirmed a significant improvement of milk thistle (silymarin) supplementation on feed intake of broilers. These explain the role of the silymarin as an anti-stress factor. This stress results in significant changes in digestibility of carbohydrates, protein, lipid, and mineral metabolism. It is speculated that supplementation of silymarin overcome the stress problems in birds, probably due to the reduction in oxidative stress (Chand et al., 2011). Also, Abdalla et al., (2018) found that milk thistle supplementation (at 25 g/kg diet) significantly increased in feed intake. While Ondrej et al., (2015) reported the lowest feed consumption in the group fed on 15% of silymarin, which was lower live weight of chickens. Therefore, it seems that a selected relatively high percentage of milk

thistle seed cakes worsen FI. This may be due to the content of substances with a bitter taste.

In addition, the significantly improved were showed in feed conversion ratio of the birds in fourth group (table 4). The results were in agreement with those used silymarin supplementation to the diet and improved nutrient digestibility and feed efficiency in broiler broilers and increase body weight as reported by (Saller et al., 2007), who concluded that nutrient digestibility was enhanced in broilers supplied with silymarin due to an improvement in gut health. The decrease in body weight, weight gain, feed intake and in feed conversion ratio could be due to the fact that the mycotoxins interferes with all of the metabolic processes in the body and inhibits protein synthesis necessary for growth (Fouad et al., 2019).

The results of this study were in agreement with the findings of (Tedesco et al., 2004), who observed better feed efficiency response (2.527) in AFB1 contaminated feed with milk thistle with respect to birds receiving AFB1 contaminated feed alone (2.929), during the last two weeks. Similar results were also reported by (Morovat et al., 2016). Zahid and Durrani, (2007) denoted that supplementation milk thistle to broilers diet at rate of 15 g/kg feed led to improved FCR in treated groups as compared with control group. Also, cockerels fed on diet supplied with silymarin which recorded improved significantly of FCR (Abdalla et al., 2018).

**Hematological parameters:** The haematological parameters showed significant ( $P \leq 0.01$ ) differences among treatments and control groups. Data of table (5) revealed significant ( $P \leq 0.01$ ) decreased in WBCs count, RBCs count, PCV% and Hb concentration of the second and fourth groups as compared with first and third groups, while, the Hb concentration was significant ( $P \leq 0.01$ ) increase in the fourth group as compared with second group. These results were related to those findings by (Celik et al., 2000; Elaroussi et al., 2006 and Pande et al., 2006) when chicks fed on contaminated feed with mycotoxins. However, the effect of mycotoxins on RBCs and, WBCs counts cause anemia. Anaemia characterized by a significant decrease in PCV and Hb concentration levels was reported and attributed to iron deficiency or as a consequence of a disturbance in the haemopoietic system (Elaroussi et al., 2006). The decrease in a number of leucocytes was reported to be a reflection of a decrease primarily of lymphocytes and to a lesser extent monocyte (Chang et al., 1979) or heterophils (Chang et al., 1981 and Mohiuddin et al., 1993). Such a lymphocytopenia may be a sensitive and useful indicator of mycotoxins that possibly may occur due to a direct

effect on germinal centers of lymphoid tissues and implies alteration of the immune function. The effects of mycotoxins on blood cells caused damage of the cells which leads to decrease in the quantity of the blood and decrease in Hb concentration, these results agreed with (Mohiuddin *et al.*, 1986) who reported The effect of AFB1 cause narcotization and hemorrhage in the digestive tract which leads to decrease in the quantity of the blood and decrease in Hb concentration.

In the current study, there is a significant relationship between mycotoxins and decrease in haematological parameters. Mycotoxins inhibit production and decrease the life span of RBCs and reduce the Hb level. The haematological parameters showed a significant reduction in the broilers fed on the diet contamination with mycotoxins. This might be linked to the toxin with blood cells caused death of the cells and then break up of these cells, the toxin also causes decrease in Hb. Also, stressed hormones (corticosteroids) have suppressive effects on total leucocytes count. This result agreed with (Hamood, 2013 and Naseem *et al.*, 2018).

However, silymarin supplementation effect on the total Hb concentration. These results agreed with Ajay *et al.*, (2009) who found the ability of silymarin to improving the absorption of iron. Also, Sultan *et al.*, (2018) reported that enhancement of haematological parameters in the present of silymarin. Talebi *et al.*, (2015) found that Silymarin significantly helps to keep the levels of haematological and serum biochemical parameters in normal range. It can be used effectively to reduce the toxic and suppressive effects of mycotoxicosis.

### Conclusions

The research study concluded that 0.5% silymarin / kg feed could be increase in average body weight, weight gain, feed consumption and better feed conversion efficiency for broiler chicks at the end of the experiment. As well as a significant rise in a number of RBCs, WBCs count, PCV ratio, Hb concentration.

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