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# Effect of Adding Milk Thistle *Silybum marianum* Cereal to Naturally Contaminated Ration by Mycotoxins on Productive and Physiological Performance of Laying Hens.

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**ABSTRACT**

The aim of the current study was to evaluate the effect of adding different levels of milk thistle cereal powder to the ration naturally contaminated by mycotoxins on productive and physiological performance of laying hens fed. A total of 108 laying hens at 49 weeks old were used in this study. Birds were randomly distributed to four treatments each treatment with three replicates and nine birds in each replicate. The experimental treatments were as the following :Treatment 1 (Negative control) fed standard ration, treatment 2 (Positive control) fed standard ration its content yellow corn was naturally contaminated by Mycotoxins (aflatoxins 87.58 ppb,T-2 toxin 1.13 ppb and Ochra Toxin 2.0 ppb ), treatment 3 fed as treatment 2 + Milk thistle 7.5 gm/kg feed, treatment 4 fed as treatment 2 + Milk thistle 15 gm/kg feed. The experimental period was 112 days. The results obtained showed there was supplementation of milk thistle of contamination ration alleviated the adverse effects of mycotoxins significantly ($P \leq 0.05$) on egg prodution , egg weight, egg mass and feed convertion ratio, as well on blood biochemical (Immunity, Protein, ALT , AST , Cholesterol) and histological indices of intestinal (Villi high, Crypts depth), while the experimental treatments had no significant effect on the concentration of uric acid in the blood. Results from this study suggested that adding of milk thistle to the contamination ration by mycotoxins alleviated the adverse effects of mycotoxins on productive and physiological performance of laying hens.

**Keywords:** Milk Thistle ; Mycotoxicosis; Laying hen; Productive; Physiological.

## INTRODUCTION

Contamination of poultry diet by mycotoxin is one of the major challenges related with poultry industry[1]. Mycotoxins are toxic compounds, biologically active with low molecular weight, naturally produced by metabolism of some types of fungi, such as, Aspergillus, Fusarium , and Penicillium species [2, 3] . Many types of mycotoxins such as aflatoxin, Ochratoxin A , T-2 toxin , zearalenone, , vomitoxin, and fumonisins, naturally contaminate feed materials [4]. Some factors are necessary for fungi to generate mycotoxins , including high temperature and humidity , physical condition of grain , storage time, and presence of fungi in grain [5]. Mycotoxins are found in all types of cereals which are used in food of animal and human, mainly in corn, and cause vast eco-nomic losses through reducing birds performance, health and immune response [6, 5]. Mycotoxins cause a wide variety of adverse clinical signs, depending on the concentration and nature of toxins in the diets, the animal species, their age, and health status at the time of exposure to contaminated diet[7] . High concentration of mycotoxins in diet results the appearance of acute mycotoxicoses and high death rate while lower concentration cause the chronic mycotoxicoses without showed clinical signs, but followed by the considerable decrease in production performance, health status, and immunosuppressive effects[8] . Due to the high cost of poultry feed, which represent more than 70% of cost of production chain, it is necessary to reduce or eliminate mycotoxin in animal feed, many natural, physical, biological and chemical methods are used for this purposes[9] . Unfortunately, all these methods significantly reduced amount of mycotoxin , but they do not eliminated it completely[7] .

Recently, medical plants have received a lot of attention as feed additives for elimination the adverse effects of mycotoxins, milk thistle (*Silybum marianum*) is one of the main medicinal plants

that commonly used for treatment of liver disease, different flavonolignans , such as silybin are present in milk thistle extract, the main bioactive compound in milk thistle are present in seeds, which contains about70%-80% Silymarin, in addition to its hepato-protective effect, silymarin acted as antioxidant, anti-inflammatory, antifibrotic, anti-lipid peroxidative, cell membrane stabilization and liver restoring effects[10]. Metabolically, Silymarin stimulates the hepatic cells and induces the synthesis of ribosomal RNA to promote protein production[11]. The aim of the current research was evaluating the influence of supplementation diferent levels of milk thistle *Silybum marianum* cereal powder on the productive and physiological performance of laying hens fed a ration naturally contaminated by mycotoxins.

## MATERIALS AND METHODS

One hundred and eight, age 49 weeks, laying hens (Lohmann white) were used in the study. Layer hens were randomly divided into four treatments of 27 birds. Each treatment with three replicates.The hens were reared in floor cages 1.5×1m(9 birds/ pen).The birds were fed on the following treatments: T1 (Negative control) fed on free mycotoxin basal diet (Table 1): T2 (Positive control ) fed on basal diet its content yellow corn was naturally contaminated by mycotoxin.T3 fed as T2 + Milk thistle cereal powder 0.75 gm/ kg feed, T4 fed as T2 + Milk thistle cereal powder 1.5 gm/ kg feed. Naturally contaminated yellow corn by mycotoxins was obtained from private feed factory, and examined at master lab. (Netherlands), and recorded these results: aflatoxins 87.58 ppb, T-2 toxin 1.13 ppb and Ochra Toxin 2.0 ppb. Milk thistle cereal were obtained from the local market, and ground before being added to the diet. The lighting program was 16 hour per day. Drinking Water was *ad libitum*, whereas diet was provided constrained (110 gm/day/bird) during the experiment period. The duration of the experiment lasted 122 days at the age of 49-64 weeks.

Egg production (H.D%) were documented daily, egg weight, egg mass, feed conversion ratio (FCR) were recorded weekly. At the last day on the experiment (age 64 week), 12 layer hens were slaughter (3 birds from each treatment), Blood samples were collected by jugular vein of the neck and placed in non-additive blood collection tubes to produce serum blood samples were collected randomly in nonheparinized tube. Measure of biochemical blood serum, serum was separated by centrifugation at 1800g, for 15 minutes and analysed Newcastle titers using ELISA technique, uric acid, total protein, ALT, AST, and cholesterol by a biochemical analyser using commercially available kits (Spanish company SPINRECT). After extracting the birds intestine, histological examination was conducted, as a sample of the jejunum two cm was taken for each of the treatment birds, they were washed with physiological saline. Then, the samples were placed in formalin 10%, and then the tissue slides were prepared according to[12]. All the prepared slides were examined using a light microscope at a magnification 40x. Measurements were recorded using the ocular micrometer after calibrated with the stage micrometer. The villi length and crypts depth were both estimated, where the villi length extends from the tip of the villi to its attachment to the crypt, and the crypt depth is the distance from the base of the villi to the end of the crypt [13] were determined.

Statistical analysis of data by using complete random design (CRD ), a Duncan's multiple range test[14] was used to test the significance of the differences between the treatment averagesl at level of probability $P \leq 0.05$. Statistical analysis were performed using SPSS software.

**Table 1.** Composition and calculated chemical analysis of the basal experiment diets used in the study.

| Feed ingredients | (%) |
|---|---|
| **Yallow Corn** | 66.5 |
| **Soybean meal** * | 22 |
| **Premix**** | 2.5 |
| **limestone** | 8 |
| **Dicalcium phosphate** | 1 |
| **Total** | 100 |
| **Calculated chemical analysis** *** | |
| **Crud protein (%)** | 15.833 |
| **Metabolizable Energy (kcal/ kg)** | 2823.05 |
| **Methionine (%)** | 0.418 |
| **Methionine+Cystine (%)** | 0.716 |
| **Lysine (%)** | 0.840 |
| **Calcium (%)** | 3.441 |
| **Available phosphorus (%)** | 0.520 |

* Soybean meal contain: 44% crud protein and 2230 kcal/kg metabolizable energy.

**Dutch Nuscience premix contain: 40% crud protein , Lysine 3.85% , Methionine 3.70 , Methionine +Cystine 3.9 , Calcium 6.00% , available phosphorus 3.00% and metabolizable energy 4188 kcal/kg.

***Calculated according to NRC[15].

## RESULTS

### Productive performance

Results of Egg production (H.D%) , Egg weight, (g), Egg mass (g/hen/day) and Feed conversion ratio (g feed/ g egg mass) are presented in Table 2. According to this table, the productive performance parameters decreased in group fed mycotoxins contaminated diets (T2) compared with control (T1) , while, supplementation of contaminated diets with different levels of milk thistle seeds (7.5, 15 g /kg feed) (T3 and T4 respectively ) alleviated the adverse effects of mycotoxins on productive performance. Egg production (H.D%), Egg weight, (g), Egg mass (g/hen/day) and feed conversion ratio (FCR) were significantly lower ($p \leq 0.05$) in groups fed mycotoxins contaminated diets T2 compared with control (T1 ).

**Table 2.** Effect of adding Milk Thistle Silybum marianum cereal to naturally contaminated ration by mycotoxins on productive performance of laying hens (mean ± standard error).

| Item | Treatments | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | Sig. |
| Egg production (H.D%) | 72.91±0.21[a] | 67.93±2.40[b] | 72.85±0.17[a] | 72.95±0.15[a] | * |
| Egg weight (g) | 62.85± 0.27[a] | 61.04±1.07[b] | 63.17±0.17[a] | 63.24±0.24[a] | ** |
| Egg mass (g/hen/day) | 45.77±0.32[a] | 41.54±2.17[b] | 46.02±23[a] | 46.14±.27[a] | * |
| Feed conversion ratio (g feed/ g egg mass) | 2.10±0.05[b] | 2.37±0.13[a] | 2.06±0.01[b] | 2.06±0.01[b] | ** |

T1 (Negative control) fed standard ration, T2 ( positive control ) fed standard ration its content yellow corn was naturally contaminated by Mycotoxins, T3 and T4 fed as treatment 2 + Milk thistle 0.75, 1.5 gm/ kg feed respectively.

* and ** Significant effects at the probability level P≤0.05, P≤0.01 respectively.

a-b within one column indicated significant differences between the averages at level P≤0.05 in the Duncan Multiple Range Test.

**Serum biochemistry and some liver enzymes**

The effects of mycotoxins and different levels of Milk thistle powder seeds on serum biochemical parameters are shown in Table 3. The results showed that newcastle disease Virus titer (NDV) showed a significant reduction (p≤ 0.05) in birds fed mycotoxins contaminated diets (T2) compared with control group (T1), the birds fed mycotoxins contaminated diets containing about 7.5 and 15 gm/kg feed milk thistle (T3 and T4) did not show asignificant change in titer of NDV compared to control group (T1). There was no significant differences in concentration of Uric acid in all treatments of the experiment. Feeding mycotoxins contaminated diets caused significant decrease (p≤0.05) in concentration of total protein compared with the control group (T1). In the group feed mycotoxins contaminated diet (T2), the concentration of ALT , AST and Cholesterol was higher significantly (p≤0.05) compared with the control group (T1). Concentration of total protein , ALT , AST . and cholesterol did not significantly differs (p≤0.05) in treatments containing different levels of milk thistle seeds T3 and T4 (7.5 and 15 g/kg feed) compared to control group T1.

**Table 3.** Effect of adding Milk Thistle Silybum marianum cereal to naturally contaminated ration by mycotoxins on serum biochemistry of laying hens(mean ± standard error).

| Item | Treatments | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T2 | T3 | Sig. |
| Titer NDV. | 11191±452.10 [a] | 7868±226 [b] | 11291± 671.2[a] | 11316±397.07[a] | ** |
| Uric acid (mg/dl ) | 5.93±0.08 | 6.13±0.33 | 5.93±0.8 | 5.93±0.6 | N.S |
| T. Protein (g/dl) | 6.36± 0.27 [a] | 5.00±0.26[b] | 6.63±0.20[a] | 6.51±0.19[a] | * |
| ALT U/L | 29.00±0.57[b] | 60.90±2.0[a] | 30.73±2.16[b] | 30.20±2.37[b] | * |
| AST U/L | 35.23±0.92[b] | 60.60±2.38[a] | 38.26±1.96[b] | 36.33±1.7 [b] | * |
| Cholesterol (mg/dl) | 127.33±12.5[b] | 183.00±13.79[a] | 127.33±12.5[b] | 135.66±1.4[b] | * |

T1 (Negative control) fed standard ration, T2 ( positive control ) fed standard ration its content yellow corn was naturally contaminated by Mycotoxins, T3 and T4 fed as treatment 2 + Milk thistle 0.75, 1.5 gm/ kg feed respectively.

N.S. represents no significance.

* Significant effects at the probability level P≤0.05.

a-b within one column indicated significant differences between the averages at level P≤0.05 in the Duncan Multiple Range Test.

**Histological indices of intestinal**

As shown in table 4. the Results of statistical analysis of Villi height and Crypts depth showed a significant reduction ($p \leq 0.05$) in birds fed mycotoxins contaminated diets (T2) compared with control group (T1) . The birds fed mycotoxins contaminated diets containing about 7.5 and 15 gm/kg feed milk thistle (T3 and T4) did not show asignificant change in villi height and crypts compared to control group (T1).

**Table 4.** Effect of adding Milk Thistle Silybum marianum cereal to naturally contaminated ration by mycotoxins on histological indices of intestinal (mean ± standard error).

| **Item** | **Treatments** | | | | |
|---|---|---|---|---|---|
| | T1 | **T2** | **T3** | **T4** | **Sig.** |
| Villi hight (µm) | 1094.00±23.86[a] | 836.67 ±12.01[b] | 1033.00±21.65[a] | 1050.00±28.86[a] | ** |
| Crypts depth (µm) | 241.67 ± 1.66 [a] | 166.66 ±12.73[b] | 225.44±3.o8 a | 229.07±3.55 [a] | ** |

T1 (Negative control) fed standard ration, T2 ( positive control ) fed standard ration its content yellow corn was naturally contaminated by Mycotoxins, T3 and T4 fed as treatment 2 + Milk thistle 0.75, 1.5 gm/ kg feed respectively.

** Significant effects at the probability level $P \leq 0.01$.

a-b within one column indicated significant differences between the averages at level $P \leq 0.05$ in the Duncan Multiple Range Test.

**DISCUSSION**

Mycotoxins contaminated diet alone caused lower egg production, egg mass , egg weight.and that reflected negatively on fed conversion ratio (FCR) due to the negative effect of mycotoxine. The reduction in productive performance due to mycotoxins effect in the current study may be attributed to many factors :- firstly , the hepatotoxic effects of mycotoxins suppressing protein synthesis through reduced functionality of enzymes and substrates, secondly mycotoxins interferes with DNA, RNA and protein synthesis and affects carbohydrate metabolism, particularly glucogenolysis[16]. Moreover, the effect of aflatoxins in reduction villus hight and absorptive surface area, causing reduced in nutrient absorption , that affect on productive performance [17] as confirmed by this study(table 4).

Adding milk thistle into mycotoxins contaminated diets alleviated the adverse effects of mycotoxin on productive performance of laying hens, due to the multiple actions of milk thistle and its active compound (Silymarine ) wich act as hepatoprotective agent and has antioxidant activity that stimulated protein synthesis by the bird's enzymatic system, and prevent it's damaging in liver cells[18] as confirmed by this study(table 3).

The lower Newcasle disease titers were recorded in groups fed aflatoxin b1 contaminated diets compared to control group. It was clear that mycotoxins caused severe immuno-suppression due to reduction in phagocytic activity of blood monocytes, depressed complement activity, hence depressed opsonization and phagocytic activity[8]. However, treatment with milk thistle protected the reduction of humoral immune response in layer hens due to mycotoxins in feed [19]. Milk thistle supports the immune system through its powerful antioxidant, free-radical scavenging action, its ability to preserve the supply of another important antioxidant, glutathione, as well as direct effects on immune cells [20].

The decreas in serum total protein in birds fed mycotoxins contaminated diets may be due to inhibition of protein synthesis that happened by competitive inhibition of phenylalanine-t-RNA-synthesis[16]. supplementation different levels of milk thistle to mycotoxins contaminated diet improve the total protein concentration. This improvements was related to the role of adding milk thistle that contain compound named silymarine which have antioxidant properties prevent the free radical-induced hepatocytes damage to liver cells. Silymarin also promotes liver cell protein synthesis and decreases the oxidation of glutathione [19].

In the current study , the AST and ALT levels in mycotoxins treated birds increased significantly compared with control birdsIt was evident from the findings that mycotoxins caused liver damage and leakage of enzymes, resulting in elevated ALT and AST levels. However, supplementation of birds feed with milk thistle seeds prevented the rise in values of ALT and AST by keeping liver healthy due to its anti-oxidative property[16, 21].

The lower cholesterol concentration was observed in birds fed mycotoxins contaminated diet. mycotoxins are stressful and the stress in birds causes cortisone to be secreted by the adrenal cortex, which has negative feedback on thyroid activity, causing an increase in cholesterol and triglycerides[16]. Birds fed mycotoxin diet with 7.5 and 15 gm/kg milk thistle showed an enhancement in cholesterol concentration .This enhancement was related to active compounds of milk thistle which has antioxidant properties and protective effect against mycotoxin[20].

The decreased villi height and crypt depth recorded in mycotoxins contaminated dietsgroup may be related to impairments in epithelial protein synthesis and decrease in cell proliferation[16]. Supplementation different levels of milk thistle to mycotoxins contaminated diet enhanced the villus height and crypt depth in the jejunum of laying hens, the observed effects of milk thistle on intestinal morphology may be resulted from the removal of invasive pathogenic microorganisms that shifts the bacterial community towards the predominance of the fermentative taxa [19 , 22, 23].

The groups fed mycotoxind diet containg different levels 7.5 and 15 g/kg feed of milk thistle was non-significantly different compared with the control group. It indicate amelioration from negative effect of mycotoxins with milk thistle. The findings of the current study are supported by many studies such as studies of [24-27], whose found that milk thistle and its extract significantly help in keep the levels of serum biochemical parameters in normal range. it can be used efficiently to decrease the toxic and suppressive effects of mycotoxicosis.

## CONCLUSIONS

Adding 7.5 or 15 g/kg feed milk thistle seeds into naturally contaminated diets showed enhancement in productive performance and blood biochemical Parameters. indicating a protective effect of milk thistle against mycotoxin . Milk thistle may be used at level 7.5 mg/kg feed for ameliorating the adverse effects of mycotoxin on Productive Performance and serum blood biochemical parameters of laying hens.

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**Conflicts of Interest:**

The author declares that no conflict of interest exists

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