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Clinicopathological Studies on the Effect of Fusarium Mycotoxin on Hematological and Biochemical Parameters in Broiler Chickens

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Abstract: Mycotoxins are produced by fungi. Trichothecenes are a group of mycotoxins mainly produced by Fusarium species. The present study was conducted on sixty, one day old broiler chicks. The chicks were equally divided into three groups (Gps). Gp. (1) was the normal healthy control. Gp. (2) received fusarium (10 mg/kg diet) from 1-28 days old. Gp. (3) received fusarium (10 mg/kg diet) and antimycotoxin { (T-Nil® plus) liquid (0.25 ml/liter)} from 1-28 days old. Chickens received fusarium toxin showed a significant decrease in both body weight and gain. Erythrogram revealed a normocytic normochromic anemia. Leucopenia, heteropenia and lymphopenia were pronounced. Biochemical studies revealed s significant changes in the hepatic and renal function tests. There was a significant increase in phosphorus with hypocalcemia. The administration of antimycotoxin significantly improved the body performance, hemogram and biochemical parameters compared with fusarium toxin received group. It could be concluded that fusarium toxin produces negative impacts on the performance, hemogram, liver and kidney of the chickens. The antimycotoxin (T-Nil® plus) can ameliorate the effect of fusarium toxin.

Key words: Biochemical · Fusarium · Hematology · Mycotoxins · T-Nil® Plus

INTRODUCTION

Recently the growing economic value of poultry industry all over the world and the subsequent expansion of this industry have been accelerated by the increased researches on the avian diseases. The problems, associated with mycotoxins, constitute a great hindrance for the poultry industry. Mycotoxins are produced by fungi, which has the ability to contaminate foods and produces mycotoxicosis [1]. Mycotoxicosis is characterized as feed-related, non- transferable and non-infectious diseases [2]. Trichothecenes are a group of mycotoxins mainly produced by Fusarium species. Deoxynivalenol is one of the most important trichothecenes in food as it has high frequent occurrence in toxicologically relevant concentrations worldwide [3]. Since toxin production depends on environmental conditions, such as temperature and humidity, fusarium toxin contamination cannot be avoided completely. It is present in all kinds of grains, such as wheat, rye, barley and oats [4]. The structure of deoxynivalenol is stable and resists low pH levels[5]. Deoxynivalenol is the most prevalent fusarium toxin in poultry feed. The acute form of deoxynivalenol toxicity rarely occurs in poultry flocks under normal conditions. However, if diets contain low levels of deoxynivalenol, lower productivity, impaired immunity and higher susceptibility to infectious diseases can occur [6]. Ingestion of mycotoxins causes a wide range of toxic responses, from acute toxicity to long-term health disorders. Antimycotoxin act as a bio-toxicosis product revitalises poultry that suffering from clinical and sub-clinical signs of toxin-related health problems, depending on its ability to binding the toxin. Adsorbents effect of the antimycotoxin based on yeast cell wall from Saccharomyces *cerevisiae* which contain esterified glucomannan, as an alternative to reduce the mycotoxins bioavailability [7]. In addition to antimycotoxin components as organic acids and copper sulphate which destroy the molds and their spores.

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MATERIALS AND METHODS

Chicks: The present study was conducted on sixty, one day old broiler chicks. The birds were housed in proper hygienic conditions, maintained on a commercial well balanced ration and water ad libitum. The chicks were vaccinated against Newcastle disease [8].

Fusarium Mycotoxin

Fusarium Species: Fusarium graminearum, kindly provided from Animal Health Lab, El-Doky, Egypt.

Fusarium Mycotoxin: Was produced by the inoculation of Fusarium graminearum on wet corn grains as described by Altpeter and Posselt [9]. Fusarium containing corn was quantitatively evaluated using Thin Layer Chromatography technique (TLC) according to Thrane [10]. The toxin is a very stable compound, both during storage, milling and the processing, cooking of food and it does not degrade at high temperatures [11].

Antimycotoxin

T-Nil® plus: for prevention of mycotoxicosis in the feed stuff. It is a high quality product which physically adsorbs the polar mycotoxins and contains neutral fermented extraction for biotransformation of less and none polar mycotoxins. It takes two forms; one for feed additive (New Toxy Nil-Dry) and the other for drinking water (T-Nil-Plus liquid), Nutriad Company, Belgium.

Contents of Antimycotoxin: Fermentation extracts of Saccharomyces cerevisiae, citric acid, lactic acid, phosphoric acid, aspartic acid, propylene glycol, Vitamin B1,2, Biotine, isoleucine, copper sulphate, sodium, calcium, magnesium, sulfur and phosphorous.

Experimental Design: The chicks were equally divided into three groups. Gp. (1) was the normal control. Gp. (2) received fusarium mycotoxin (10 mg/kg diet) from 1-28 days old. Gp. (3) received fusarium mycotoxin (10 mg/kg diet) and antimycotoxin [T-Nil® plus liquid (0.25 ml/liter)] from 1-28 days old.

Evaluation of Growth Performance: The birds were weighed individually at one day old to obtain the initial body weight. The body weight was recorded weekly to calculate the average body weight in each group. The body gain was calculated every week [12].

Blood Sampling: Two blood samples were collected at the same time, by heart puncture, at the end of the 4th week. The first blood sample (0.5ml) was collected on disodium salt of EDTA and used for hematological examination. The second blood sample (5 ml) was collected in a centrifuge tube to separate serum for determination of the serum biochemical assays.

Hematological Studies: Erythrocytic (RBCs) and total leucocytic counts (TLC) were performed using the improved Neubauer hemocytometer and Natt and herrick solution as special diluents. The packed cell volume (PCV) was estimated by the microhematocrit centrifuge [13]. The hemoglobin (Hb) estimation was performed using the cyanmethemoglobin colorimetric method after centrifugation [14]. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated. The differential and absolute leucocytic counts were carried out [13].

Biochemical Studies: Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities [15] and lactate dehydrogenase (LDH) [16] were measured. Serum total protein (TP) [17], albumin [18] were determined and the serum globulins were calculated as the difference between TP and albumin. Serum levels of creatinine [19], uric acid [20], calcium (Ca) [21] and inorganic phosphorus [22] were estimated..

Statistical Analysis: The data were analyzed by using SPSS for Windows. Data are shown as mean ± Standard Error. The significance of differences was calculated by using one-way analysis of variance (ANOVA) followed by Dancan procedure for multiple comparisons. Any value of $P \leq 0.05$ was considered significant. Means at the same column followed by different letters were significantly different and the highest value was represented with the letter a [23].

RESULTS AND DISCUSSION

This study was conducted to illustrate the impact of fusarium toxin on chickens performance, hemogram and biochemical parameters. Chickens feed on mycotoxin shown feed refusal, reduced feed intake, diarrhea and abnormal wing positioning. In turn, chickens received fusarium toxin (Table 1) proved a significant decrease in both body weight and gain. This may be a reflection of decreased feed intake, decreased utilization or negative

Table 1: Body weight and gain of chickens in different groups (mean values ± SE)

Gps	Body weight/gm/ chicken					Body weight gain/gm/chicken			
	One day	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
1	36.40±1.09 a	145.78±2.76 a	335.59±4.96 a	721.52±10.66 a	1077.40±2.45 a	109.38±3.35 a	189.80±2.70 a	385.93±5.70 a	355.87±4.07 a
2	35.30±0.83 a	98.40±9.99 b	237.58±3.30 b	523.62±4.15 b	810.45±7.09 b	63.10±10.06 b	139.18±11.43 b	274.25±4.12 b	286.83±7.79 b
3	35.90±1.06 a	143.94±3.16 a	330.15±7.34 a	709.82±15.78 a	1059.71±2.93 a	108.04±2.80 a	186.21±4.49 a	379.67±8.44 a	349.89±4.89 a

Gp. (1) normal control Gp. (2) fusarium mycotoxin,

Gp. (3) fusarium mycotoxin and T-Nil® plus.

Different letters significantly different at P≤ 0.05.

Table 2: Erythrogram of the chickens in different groups (mean values ± SE)

Gps	RBCs (x 10^6/µl)	PCV%	Hb gm/dl	MCV fl	MCHC %
1	4.66±0.03 a	34.50±0.78 a	11.57±0.21 a	73.99±1.65 a	33.73±1.13 a
2	4.16±0.04 b	31.20±0.59 b	11.24±0.41 b	75.02±1.70 a	36.04±1.20 a
3	4.62±0.09 a	33.60±0.99 a	11.59±0.26 a	72.66±1.56 a	34.86±1.56 a

Gp. (1) normal control

Gp. (2) fusarium mycotoxin,

Gp. (3) fusarium mycotoxin and T- Nil® plus.

RBC red blood corpuscles

Hb hemoglobin

PCV packed cell volume

MCV mean corpuscular volume

MCHC mean corpuscular hemoglobin concentration

Different letters significantly different at P≤ 0.05.

Table 3: Leucogram of chickens in different groups (mean values ± SE)

Gps	TLC(x10^3/µl)	Heterophil(x10^3/µl)	Lymphocyte(x10^3/µl)	Monocyte(x10^3/µl)	Eosinophil(x10^3/µl)
1	22.72±0.41 a	8.58±0.22 a	12.74±0.23 a	0.60±0.03 a	0.52 ±0.08 a
2	19.71±0.46 c	7.51±0.29 b	10.75±0.32 b	0.59±0.05 a	0.47 ±0.04 a
3	22.02±0.45 a	8.36±0.31 a	12.39±0.26 a	0.57±0.05 a	0.47 ±0.04 a

Gp. (1) normal control

Gp. (2) fusarium mycotoxin,

Gp. (3) fusarium mycotoxin and T- Nil® plus

TLC total leucocytic count

Different letters significantly different at P≤ 0.05.

Table 4: Some liver function tests in the serum of chickens in different groups (mean values ± SE)

Gps	AST(U/L)	ALT(U/L)	LDH(U/L)	Total protein(gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)
1	76.07±0.77 b	14.87±0.46 b	81.55±1.47 b	4.21±0.09 a	2.52±0.05 a	1.68±0.03 a
2	88.45±1.26 a	21.63±1.30 a	95.09±1.72 a	3.64±0.11 b	2.18±0.06 b	1.45±0.04 b
3	79.31±1.17 b	15.58±0.49 b	85.72±1.65 b	4.05±0.18 a	2.43±0.06 a	1.62±0.04 a

Gp.(1)normal control Gp.(2)fusarium mycotoxin,

Different letters significantly different at P≤ 0.05.

Gp.(3)fusarium mycotoxin and T-Nil® plus

AST aspartate aminotransferase

ALT alanine aminotransferase

LDH lactae dehydrogenase

Table 5: Some renal function tests in the serum of chickens in different groups (mean values + SE)

	Parameters			
Gps	Creatinine (mg/dl)	Uric acid (mg/dl)	Ca (mg/dl)	P (mg/dl)
1	0.77±0.04 [c]	6.43±0.12 [b]	11.69±0.19 [a]	5.63±0.09 [b]
2	1.03±0.04 [a]	6.89±0.08 [a]	10.17±0.18 [b]	6.15±0.11 [a]
3	0.89±0.03 [b]	6.68±0.13 [ab]	11.26±0.22 [a]	5.74±0.10 [b]

Gp. (1) normal control

Gp. (2) fusarium mycotoxin, Gp.

Gp. (3) fusarium mycotoxin and T- Nil® plus

Ca: calcium

p: phosphorus

Different letters significantly different at $P \leq 0.05$.

impact on intestine and liver. This supports the previously obtained results as Rotter *et al*., Awad *et al.* and Bergsjo *et al*., [24-27] recorded a significant decrease in body weight and gain of chickens received fusarium toxin.

The erythrogram (Table 2) revealed a significant decrease in the RBCs count, packed cell volume and hemoglobin concentration, beside non significant change in the mean corpuscular volume and mean corpuscular hemoglobin concentration. This normocytic normochromic anemia may be due to decrease of the life span of RBCs or suppression of the bone marrow stem cell activity [13, 27- 28]. The observed leucopenia (Table 3) in fusarium treated group is due to heteropenia and lymphopenia[27]. The decrease in the heterophils and lymphocytes may be due to the negative impact on bone marrow and lymphoid tissues [13, 29]. Previous results obtained by Lun *et al*., [30] indicated that fusarium toxin shown to has a cytotoxic effect and preventing the cell division in bone marrow and lymphoid tissues.

In regard to the biochemical studies conducting during this work, significant changes in the hepatic and renal function tests were observed. The hepatic biomarkers (aminotransferases and lactate dehydrogenase) (Table 4) were significantly increased in fusarium toxin treated group when compared with normal control group. The increase in the hepatic biomarkers indicates presence of liver damage [29, 31]. The proteinogram (Table 4) of treated group revealed a significant hypoproteinemia, hypoalbuminemia and hypoglobulinemia in the fusarium received group[27].This may attributed to the disturbances of metabolism by the liver, in addition to the effect of fusarium toxins on kidney which leads to albuminuria. Fusarium mycotoxin may inhibit the protein synthesis at the ribosomal level whereby rapidly proliferating cells in tissues with high protein turnover rates [32]. This confirmed with obtained result by Lun *et al*., [30] who reported hepato-renal damage in chickens received fusarium toxin.

The present work showed that the fusarium toxin damaged the renal tissue as clarified by biochemical parameters (Table 5). This renal damage was reflected by an increased serum uric acid, creatinine and phosphorus with hypocalcemia [27]. Hyperurecemia, in birds, occurs with starvation, gout, massive tissue destruction and renal diseases [33]. Some investigators think that the serum creatinine may become elevated in birds with renal diseases, but less reliably than the uric acid [34]. Hyperphosphatemia may be due to a decrease in the calcium level and an increase of the parathormone hormone as a result of hypocalcemia. The latter may be attributed to a reduced renal calcium reabsorption, decreased calcium absorption from the intestine, increased excretion and /or hypoalbuminemia [33]. Such biochemical changes, in the present work, are the outcome of nephropathy which nearly similar to those findings reported by Saif and Engelhardt *et al*., [28, 29].

The administration of antimycotoxin {T-Nil® plus liquid (0.25 ml/liter) from 1-28 days old} significantly improved the body performance, hemogram and biochemical parameters compared with fusarium toxin received group. Where, it is physically adsorbs the polar mycotoxins. In addition, it contains neutral fermented extraction for biotransformation of less and none polar mycotoxins and contains organic acids and copper sulphate which destroy the molds and their spores [7].

CONCLUSION

It could be concluded that fusarium toxin produces negative impacts on the performance, hemogram, liver and kidney of the chickens. Also the used antimycotoxin can ameliorate the effect of fusarium toxin.

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