Egypt. J. Chem. Environ. Health, 1 (1):752-767 (2015)

The efficacy of three mycotoxin adsorbents to alleviate T-2 toxins - induced toxicity in broiler chickens

Hanaa , R. El-Hoofy

Anim. Health research Institute- Damanhur Branch

Abstract

T-$_2$ Toxin is one of the most harmful mycotoxin produced by Fusarium Spp. The present study was carried to monitor the effects of this toxin on some hematological and biochemical parameters. Three anti-mycotoxin products as adsorbents were examined including (DMSO)[Dimethyl sulfoxid, Mycosorb and Zeolite checked to study their combating activities against feed experimentally contaminated with T-2 toxin in dose 1 mg/Kg .Eighty of three day old unsexed broiler chicks were acclimated and randomly divided into Eight experimental groups (10 broiler per group) Group T1 , T2 ,T3 , were fed a basal diet supplemented with 10 ml / kg of 7% (DMSO) Dimethyl sulfoxide , 1 gm /kg Mycosorb and 1 gm /kg Zeolite respectively . T4 was fed ration supplemented with 1mg /kg T- 2 toxin .Groups T 5 , T 6 , T7 were fed T -2 toxin contaminated diet with (1mg /kg) the feed were supplemented with 10 ml / kg of 7% (DMSO) Dimethyl sulfoxid , 1 gm /kg Mycosorb and 1 gm /kg Zeolite respectively . Group T 8 represented the negative control fed on balanced died without any additives At the end of experiment birds were scarified and blood was collected on anticoagulant for hematological parameters and without anticoagulant for serum separation ad biochemical parameters .The total erythrocytes count, Hemoglobin content and total Leukocyte count were significantly decreased after T-2 toxin exposure for 6 weeks. Administration of T-2 toxin led to significant increase in serum Alanine aminotransferase, Aspartate transferase Lactate dehydrogenase, Uric acid, Creatinine and glucose . T-2 toxin produced significant decrease in serum protein, albumin, globulin, cholesterol, triglyceride, calcium, phosphorus and magnesium. Supplementation with Mycosorb .Zeolite significantly improved the hematological and biochemical parameters.

Introduction

The food and agriculture organization (FAO) Estimates that 25% of the world food crops are affected with mycotoxin .This toxins may exerts deleterious effects on poultry as well as human health .They have been detected from a variety of food and feed materials consumed by man , animals and poultry **Binder , et al.,(2007)** Trichothescenes group (TCT) of mycotoxin accounts for over one hundred fungal metabolite among those T-2 toxin **Bondy and Pestka ,(2000)** .T toxin (T-$_2$) is naturally occurring mycotoxin from the group of A –Trichothescenes (TCT) produced by

Fusarium Spp. **Eriksen,(2003)** Mainly before harvesting , when grain harvest have been delayed into the winter months ,or infected grain has been stored in cold conditions **Jordan ,et al., (2002)** and **Anjum, et al.,(2011)**

T-$_2$ toxin is the most cytotoxic and exerting an inhibitory effect on protein synthesis , disruption of D N A and R N A synthesis ,damage the parenchymatus organ (Liver , Kidneys) as well as The Immune ,digestive, nervous system **Garg,(2000);Xue,et al., (2010); Dimic ,et al.,(2011) and Maria ,et al.,(2013)**

Signs of (T-$_2$) toxin in poultry suppressed feed intake , growth depression , oral lesions abnormal feathering , decrease egg production , thinner egg shell, impairment hatchability **Casarin ,et al.,(2006)** and **Dimic ,et al.,(2011)**

(T-2) toxin is a non volatile, low molecular weight insoluble in water and petroleum ether but highly soluble in acetone, ethyl acetate, chloroform, dimethyl sulphoxide ethyl alcohol and methyl alcohol. It is highly resistant to heat and U V light therefore it is not inactivated in food production and processing or by autoclaving. (T-2) toxin could be inactivated by heating at 200 o C to 210 ^{o} C for 30 - 40 minutes **Marijana, et al., (2008)**

(T-2) toxin is rapidly absorbed from the intestinal tract , metabolized and eliminated about 80% within 48 hours , however, their toxic effects could be increased by enter hepatic recirculation **Agag , (2005)**

Mycotoxin cause a wide variety of adverse clinical signs depending on the nature and concentration of toxins in the diet , animal species ,age , time of exposure to contaminated feed **Demello and Macdonald (1977)**

. At the present, the most practical approach to ameliorate the deleterious effects of mycotoxin in animals consist on using adsorbents materials with diet to reduce the absorption of mycotoxin from the gastrointestinal tract. Few products showed effective in prevention the toxin effects of (T-$_2$) toxin Myco -Ad when added at 0.25% in broiler feed **Casarin ,et al.,(2006)** Organo alumino silicate have been reported adequate in reducing the toxicity of T-2 toxin in broiler **Medina ,et al.,(2010)** . Myco-Ad AZ was also efficacious in preventing the T-2 toxin in broiler **Forat and Douglas Zaveso (2013)**

The present study was carried to evaluate three anti mycotoxin adsorbents experimentally on combating the (T-$_2$) toxin effects on broilers across period of study

Materials and Methods

I Experimental animals

A total of 80 three days newly hatched unsexed broiler chicks (Ross) were obtained from a commercial hatchery .Chicks were acclimated and reared under uniformed managemental conditions feed and water ad libitum and continues light. The used starter and growing basal diets according to the standard NRC were used after testing for any residues of ($T\text{-}_2$) toxin. Chicks were randomly divided into eight groups ten for each.

II Chemicals:

II. 1- Toxin: ($T\text{-}_2$) toxin 98% purity (Myco Lab. Co. Chesterfield, Missouri 63077,USA) synthetic ($T\text{-}_2$) toxin from Sigma Chemical Company St. Louis ,Mo., USA

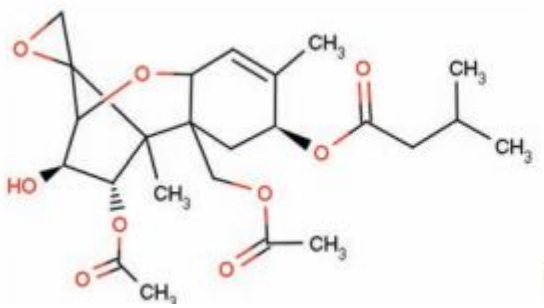

Fig. 1. Chemical structure of the T-2 toxin.

II.2- Dimethyl sulfoxid(DMOS) was obtained from El-gomhorea Company Alex. Egypt Organo-sulfur compound $(CH_3)_2$ So Polar aprotic solvent

II.3-Mycosorb was provided from Alltech, K.Y., USA. Patented broad – spectrum Mycotoxin binding feed supplement derived from yeast (cell wall glucomannan)

II.4- Zeolite: was provided by Incal Biotechnology and mining ITD, Izmir Turkey Zeiolites were hydrated Alumino-Silicate minerals.

Experimental design

This study extended for seven weeks. The broiler chicks were classified into eight groups

- ($T\text{-}_2$) toxin were feed at dose of 1 mg / kg dissolved in 10 ml of 7% Dimethyl sulfoxid (DMOS) (**Ramasamy ,et al., (2010)** -- Zeolite was added as 1 gm /Kg feed **Casarin ,et al., (2006**) Mycosorb 1 gm / Kg feed **(Ramasamy ,et al., (2010)**, 7% Dimethyl sulfoxid (DMOS) 10ml /Kg feed **Rajmon ,et al., (2001)**

Table (1) represents the dietary treatments of the eight groups under study At 45 days of age trials were terminated. Blood samples were collected into heparinized test tubes from wing vein for hematological tests parameters according to **Jain,(1986)** Another blood sample with out anticoagulant for serum separation to determine Serum aspartate amino-transferase (AST) ,Alanine amino-transferase (ALT) according to **Reitman and Frankel (1957)** Uric acid **Fosti ,et al,.(1980)** Creatinine **Henry(1974)** The concentration of serum calcium **Gindler and King(1972)** Inorganic phosphorus and Magnesium **AOAC,(1975)** Total protein **Peters ,(1968)** Albumin **Dumas** and **Biggs,(1972)** While Globulin level was determined mathematically using subs traction of albumin from total protein level .Glucose determined according to **Trinder, (1969)** Cholesterol level estimated according to **Allian et al.,(1974)** Triglyceride according to **Wahlefeld ,(1974)** Lactate dehydrogenase (LDH) according to **Heidence ,et al.,(1994)**

Statistical analyses : All data generated on performance ,hematological , and serum biochemistry of the experimental birds were subjected to statistical analyses of variance procedures of SAS Institute **SAS(2006)** The treatment means were compared using the **Ducan, (1955)** procedure of the same soft ware

Table, 1: Showing the experimental design of study the efficacy of three mycotoxin adsorbents to alleviate T2 toxins -induced toxicity in broiler chickens:

Groups	Treatment
T1	**Broiler chicks were treated with 10 ml/kg of 7% dimethyl sulfoxid (DMSO).**
T2	**Broiler chicks were treated with 1 gm. /kg of Mycosorb.**
T3	**Broiler chicks were treated with 1 gm. /kg of Zeolite.**
T4	**Broiler chicks were treated with 1.0 mg/kg of T-2**
T5	**Broiler chicks were treated with 10 ml/kg of 7% dimethyl sulfoxid (DMSO) + 1.0 mg/kg of T-2**
T6	**Broiler chicks were treated with 1 gm. /kg of Mycosorb + 1.0 mg/kg of T-2**
T7	**Broiler chicks were treated with 1 gm. /kg of Zeolite + 1.0 mg/kg of T-2**
T8	**Broiler chicks were feed on basal diet**

Results and Discussion

Mycotoxin contamination of various feed and food commodities is a global problem **Schollenberger, et al., (2006)** ($T\text{-}_2$) toxin is an important mycotoxin due to its harmful toxicity and its occurrence in grains and animal feeds. The (LD50) dose sufficient to kill 50% of 7 days old chicks is 4.97 mg/kg[1] feed . ($T\text{-}_2$) toxin is more toxic than aflatoxin (LD50) 6.8 mg l Kg [1] It is less toxic than Ochratoxin LD50 = 2.1 mg l kg **Hoerr ,et al., (1982)**

In the present study Erythrocyte count ,Leucocytic count Hemoglobin ,Packed Cell Volume (PCV) were decreased significantly ($P< 0.o5$) in the broilers feed T-2 toxin in comparison with control group Table(2) The present finding in accordance with **Pestka ,et al., (2004)**. and **Yohannes et al., (2013)** They added that ,T -2 toxin doses cause damage to bone marrow , lymph nodes , spleen .thymus leucopenia **Rizzo ,et al.,(1992**) . T-2 toxin affects the permeability of cell membranes and haemolysis of erythrocytes.

Pand ,et al., (2006) and Krishnamoorthy ,et al.,(2006) Stated that meager effect of T- 2 toxin on hematological parameters except that decrease hemoglobin and (PCV), that may be due to inhibition of protein synthesis in toxicated birds

In Table (3) significant leucocytopenia, lymphocytopenia and non significant Momocytopenia. In T-2 toxin feed group Our results were in agreement with findings of **Hoerr,(2003) ,Chowdhury ,et al., (2005**) **Yohannes et al., (2013)**

The decrease in Leukocyte (TLC) could be turned to the reduce numbers of circulating lymphocyte (Lymphocytopenia). Reduction in numbers of circulating lymphocytes could be attributed to the negative effect 0f T-2 toxin on their blast-genesis and induced DNA damage in chicken peripheral lymphocytes **Marijana ,et al., (2007)** In Table (3) Also increase percentage of heterophil count this suggests that , toxin elicited an inflammatory response **Hassan ,et al.,(2012)**) and **Yohannes et al., (2013)** Increase percentage of heterophil count might be due to the relation reduction of lymphocytes

A significant reduction in total protein ,Albumin and globulin value were observed in T -2 toxin fed birds Table(4)These results agreed with finding of **Krishnamoorthy ,et al., (2006) ; Pand ,et al., (2006) ; Moursi ,et al., (2008) and Yohannes et al., (2013)** Hypoproteinemia ,hypoalbuminemia and hypoglobulinemia observed in T-2 toxin feed group could be attributed to the reduction in feed consumption and the hepatic damage ,since , the liver is the major organ of protein synthesis especially albumin **Kaneko , et al., (1997)** Moreover **Meloche and smith (1995)** Found that T-2 toxin inhibit the protein synthesis . T-2 toxin induces DNA damage in liver tissue and

increase DNA fragmentation. The inhibitory effect of T-2 toxin have been attributed to the binding of the toxin to sub cellular components including sulphydryl groups and ribosome with ensuing inhibition of RNA ,DNA and protein synthesis **Atroshi,et al.,(1997)** In addition , **Corrier,(1991)** Stated that, T-2 toxin inhibit the protein synthesis through the inhibition of peptidyl transferase activities .

significant reduction in serum concentration of cholesterol levels table (4) probably could be attributed to depression of cholesterol biosynthesis due to the hepato toxicity **Kubena ,et al.,(1993) ; Krishnamoorthy , et al.,(2006) ; Pand ,et al., (2006)** and **Moursi ,et al., (2008)** .Also significant reduction in serum concentration of triglycried it was shown that, T -2 toxin inhibit hepatic protein synthesis causing amino acidemia as a result ,there will be greater degradation of free circulating amino acids for energy utilization , leading to excess uric acid synthesis **Meloche and Smith (1995)** This could explain the increase in uric acid level associated with feeding T -2 toxin .

In the present study feeding T -2 toxins adversely affect uric acid and Creatinine similar findings were reported by **Krishmanoorthy , et al.,(2006) ; Pand ,et al., (2006)** and **Moursi ,et al., (2008).** Decrease renal function in T-2 toxin feed chicken **Coffin and combs (1981)**

Table (4) Significant increase of AST ,ALT and LDH levels its assumed that, elevated serum enzymes level activities might indicate recent vital organs damage of T-2 toxin feed chickens these results agreed and concurrent with **Kamal -avenkatesh (2003) ; Krishnamoorthy , et al.,(2006) ; Pand ,et al., (2006) ; Moursi ,et al., (2008)** and **Yohannes ,et al.,(2013)**

Significant increase in serum concentration of glucose levels (Table 4) in Chicken fed T-2 toxin which agreed with **Yadav , et al., (2003)** On the contrary , **Rajmon et al., (2001)** assessed that ,glucose serum concentration and LDH activities dropped to nearly the significance levels in Chinese hamster treated with 1.0 mg / kg of T2 toxin

Table (5) Represented that decrease calcium, phosphorus and Magnesium levels .That reduction may be reflection of reduced feed intake **Kubena ,et al., (1998).**

$T\text{-}_2$ toxin was recorded to alter the serotonin activities in the central nervous system which is known to be involved in the regulation of appetite **Rotter ,et al.,(1996)**

Different strategies to combat Mycotoxicosis have been developed , based on the addition of adsorbents to the contaminated feed **Karaman ,et al., (2005)**

Protection from Mycotoxicosis through the use of adsorbents is economically accepted. Many adsorbents are available as hydrated alumino silicates (Zeolites);

Fibrous material from Yeast cell wall (Mycosorb) and Dimethyl sulfoxid (DMSO) organic sulpher compound . In chicken treated with Mycosorb and Zeolite (T6 ,T7) Blood parameters ; liver enzymes , Creatinine Cholesterol and Proteins were all improved when compared with $T-_2$ toxin exposed group This findings indicate mycotoxin adsorbing effects **Kubena ,et al.,(1998) and Casarin ,et al., (2006)** Recorded that , The commercial hydrated aluminum silicates (Myco-Ad - A-Z) at dose of 1.0 Kg/Mt was effective in preventing the toxic effects of T -2 toxin(1.25PPm) in broiler chicks.

Girish and Devegowda (2004) & Ramasamy, et al., (2010) Reported that Glucomannan (Mycosorb) at dose of 1 gm /Kg feed probably reduces the potency of T-2 toxin and significantly protect birds from the immune toxic effects of $T-_2$ toxin (1 PPm)**.**

In chicken treated with **DMSO** (T 5) as adsorbent alone did not significantly improve any of blood parameters tested

Parcell, (2002) Dimethyl sulfoxid (DMSO) involved in the detoxification of drugs and other harmful toxins. Moreover**, Hu, et al.,(2010)** declared that modified pine apple peel fiber in dimethyl sulphoxide novel metal ionic adsorbents

It could be concluded that feeding T -2 toxins even in minimal amount negatively affected blood parameters serum biochemical parameters. The present study clearly demonstrated that specific adsorbents could greatly diminish the toxicity of T-2 toxin in chicken The study also put recommendation that it is the time to reduce the maximum allowable limits of mycotoxin (T-2 toxin) in feed to improve animal health and the safety of the food chain

Table (2): Changes in erythrocyte counts (RBCs), Leukocyte count (WBCs), packed cell volume (PCV) , and hemoglobin content ((HB)) in the blood of broiler chickens (Ross strain) fed diet containing T-2 toxins and three mycotoxin adsorbents to alleviate T2 toxins -induced toxicity in broiler chickens after the end of experiment (42 days).

Groups	T1	T2	T3	T4	T5	T6	T7	T8
Erythrocyte count 10^6/mm^3	3.02 ^{a} ± 0.03	3.03 ^{a} ± 0.03	3.02 ^{a} ± 0.03	2.69^b ± 0.02	2.76 ^{b} ± 0.03	2.89 ^{b} ± 0.03	2.85 ^{b} ± 0.03	3.02 ^{a} ± 0.03
Leukocyte count 10^4/mm^3	26.36^a ± 2.21	26.42 ^{a}± 2.23	26.33^a± 2.20	18.35^c ±1.37	19.11^c ± 2.14	22.36^b± 1.13	22.29^b± 1.12	25.54 ^{a} ± 1.36
Packed cell volume (PCV %)	33.11 ^{a} ± 2.34	31.54 ^{a} ±2.01	34.21 ^{a} ±2.14	23.37 ^{c} ± 1.74	24.34 ^{c} ± 2.06	26.94 ^{b} ± 2.02	29.08 ^{b} ±2.08	32.56 ^{a} ± 2.42
Hemoglobin (gm. %)	11.44 ^{a} ± 1.15	10.87 ^{a} ±1.09	11.37 ^{a} ± 1.13	7.79 ^{c} ± 0.38	7.78 ^{c} ± 0.92	9.53 ^{b} ± 0.77	9.39 ^{b} ± 0.83	10.92 ^{a} ± 1.08

Within a raw, values with the same superscript letter don't differ significantly (p>0.05)

Table (3): Changes in differential leucocytic count in the blood of broiler chickens (Ross strain) fed diet containing T-2 toxins and three mycotoxin adsorbents to alleviate T2 toxins -induced toxicity in broiler chickens after the end of experiment (42 days)

Groups	T1	T2	T3	T4	T5	T6	T7	T8
Heterophils (%)	47.14 [a] ± 2.45	47.07 [a] ± 2.43	47.10 [a] ± 2.42	53.95 [c] ± 1.23	52.98 [c] ± 2.29	49.92 [b] ± 2.31	48.84 [b] ± 2.25	47.04 [a] ± 2.41
Basophils (%)	6.41 [a] ± 0.92	6.30 [a] ± 0.94	6.51 [a] ± 0.95	6.59 [a] ± 0.86	6.67 [a] ± 0.93	6.69 [a] ± 0.89	6.69 [a] ± 0.91	6.42 [a] ± 0.94
Eosinophils (%)	3.40 [a] ± 0.33	3.51 [a] ± 0.37	3.36 [a] ± 0.25	3.89 [a] ± 0.26	3.69 [a] ± 0.29	3.62 [a] ± 0.24	3.88 [a] ± 0.32	3.52 [a] ± 0.35
Lymphocytes (%)	41.14 [a] ± 1.89	41.22 [a] ± 1.78	41.19 [a] ± 1.74	33.89 [b] ± 2.88	34.96 [b] ± 2.73	37.69 [b] ± 2.89	36.70 [b] ± 2.83	41.10 [a] ± 2.88
Monocytes (%)	1.91 [a] ± 0.06	1.90 [a] ± 0.06	1.93 [a] ± 0.06	1.68 [a] ± 0.05	1.79 [a] ± 0.04	1.99 [a] ± 0.04	1.89 [a] ± 0.05	1.92 [a] ± 0.05

Within a raw, values with the same superscript letter don't differ significantly ($p>0.05$)

Table (4): Changes in glucose levels , total proteins, Albumin, Globulin, aspartate amino transferase (AST), Alanine amino transferase (ALT) , lactated dehydrogenase (LDH) activities, Urea, Creatinine, Cholesterol and Triglyceride in plasma of broiler chickens (Ross strain) fed diet containing T-2 toxins and three mycotoxin adsorbents to alleviate T2 toxins -induced toxicity in broiler chickens after the end of experiment (42 days)

Groups	T1	T2	T3	T4	T5	T6	T7	T8
Glucose (mg/L)	89.72 [c] ± 3.45	91.73 [c] ± 5.59	93.8 [c] ± 7.10	121.12 [a] ± 4.45	115.93 [a] ± 3.45	104.47 [b] ± 5.59	109.84 [b] ± 7.10	85.12 [c] ± 4.45
Total protein (g/dl)	5.37 [a] ± 0.31	5.28 [a] ± 0.29	5.66 [a] ± 0.34	2.75 [d] ± 0.17	2.95 [d] ± 0.19	3.74 [c] ± 0.20	3.39 [c] ± 0.20	4.87 [b] ± 0.28
Albumin (g/dl)	2.26 [a] ± 0.17	2.31 [a] ± 0.17	2.39 [a] ± 0.17	2.13 [b] ± 0.17	2.09 [b] ± 0.17	2.11 [b] ± 0.17	2.05 [b] ± 0.17	2.39 [a] ± 0.17
Globulin (mg/dl)	3.11 [a] ± 0.17	2.97 [a] ± 0.15	3.27 [a] ± 0.17	0.62 [d] ± 0.04	1.86 [d] ± 0.08	1.63 [c] ± 0.09	1.34 [c] ± 0.06	2.48 [b] ± 0.17
AST(IU/L)	114.69 [c] ± 3.15	119.75 [c] ± 3.15	112.55 [c] ± 3.05	145.65 [a] ± 5.15	144.47 [a] ± 3.29	127.38 [b] ± 3.58	130.18 [b] ± 4.22	112.55 [c] ± 3.75
ALT (IU/L)	14.80 [c] ± 1.11	13.96 [c] ± 1.05	14.79 [c] ± 1.11	21.55 [a] ± 2.38	20.84 [a] ± 1.78	16.33 [b] ± 1.67	16.29 [b] ± 1.68	13.84 [c] ± 1.12
LDH (IU/L)	129.90 [c] ± 6.16	125.1 [c] ± 6.66	129.2 [c] ± 6.36	189.5 [a] ± 8.38	186.43 [a] ± 7.21	155.56 [b] ± 4.78	151.32 [b] ± 4.57	127.5 [c] ± 6.32
Uric acid (mg/dl)	1.79 [c] ± 0.02	1.81 [c] ± 0.02	1.79 [c] ± 0.02	2.72 [a] ± 0.09	2.62 [a] ± 0.04	2.13 [b] ± 0.04	2.17 [b] ± 0.04	1.83 [c] ± 0.02
Creatinine (mg/dl)	0.12 [c] ± 0.01	0.12 [c] ± 0.01	0.13 [c] ± 0.02	0.92 [a] ± 0.11	0.89 [a] ± 0.08	0.71 [b] ± 0.09	0.69 [b] ± 0.09	0.11 [c] ± 0.01
Cholesterol (mg/dl)	102.66 [b] ±3.16	108.67 [b] ±3.21	109.43 [b] ±3.09	88.02 [c] ±4.16	89.96 [c] ±3.74	93.64 [c] ±3.68	94.87 [c] ±3.66	122.89 [a] ±3.23
Triglyceride (mg/dl)	123.95 [a] ±3.22	121.57 [a] ±3.16	127.38 [a] ±3.27	89.35 [c] ±2.27	93.23 [c] ±3.13	112.19 [b] ±3.23	108.25 [b] ±3.21	117.66 [a] ±3.28

Within a raw, values with the same superscript letter don't differ significantly (p>0.05)

Table (5): Changes in calcium, phosphorus and magnesium levels in plasma of broiler chickens (Ross strain) fed diet containing T-2 toxins and three mycotoxin adsorbents to alleviate T2 toxins -induced toxicity in broiler chickens after the end of experiment (42 days).

Groups	T1	T2	T3	T4	T5	T6	T7	T8
Calcium mg%	14.22 [a] ±0.23	13.39 [a] ± 0.25	13.35 [a] ± 0.24	7.28 [c] ± 0.14	7..97 [c] ± 0.17	9.33 [b] ± 0.17	9.12 [b] ± 0.17	12.57 [a] ± 0.23
Phosphorus mg %	9.33 [a] ±0.17	9.61 [a] ±0.18	9.74 [a] ± 0.18	4.98 [c] ± 0.08	5.19 [c] ± 0.06	6.58 [b] ± 0.06	6.74 [b] ± 0.06	8.98 [a] ± 0.006
Magnesium ug/g	356.45 [a] ±20.94	349.43 [a] ±20.74	351.72 [a] ± 20.86	277.96 [c] ± 16.56	288.26 [c] ± 18.76	311.56 [b] ± 18.14	305.72 [b] ± 18.36	349.54 [a] ± 20.56

Within a raw, values with the same superscript letter don't differ significantly ($p>0.05$)

Reference

Agag ,B.I.,(2005): Mycotoxin in foods and feed 5-Trichothesins T-2 toxin Assut. Univ Ball Bull. Environ. Res. Vol 8 No.2:107-124.

Allian ,C.C. ,Poo, L.S, Chan ,C.S.G ,Richmand ,W and Fu ,P.C(1974): Enzymatic determination of total serum cholesterol. Clin. Chem. 740-745.

Anjum ,M.A. ,Sahota ,A.W, Akram ,M. and Ali,I.(2011): Prevalence of Mycotoxin in poultry feed ingredients in Ben jab The J. Anim. P I Scin (21) 2 :117-120

AOAC(1975) : Association of Official Analytical Chemistry ,Official methods of analysis 12th Ed. Wasshington DC.

Atroshi ,F. Rizzo, A Biese, I. Veijalaimen ,P.. Antila ,E. Westermarck ,T.(1997): T-2 toxin induced DNA change in mouse livers the effect of pretreatment with co-enzyme Q10 alpha- Tocopherol .Molecular aspects of medicine 18 Supplemented 1):255-258

Binder,A., T**an, L.M, Chin ,L.J., Handle, J., Richard, J.(2007**):World wide occuance of mycotoxin in commodities feed and feed ingredients' Ani. Feed Sci. Technol. 137: 265-282

Bondy, G. S., Pestka, J.J (2000): Immune modulation by fungal toxins .J.of Toxicol. Environ. Health , past Bcrit Rev.3 :109-143

Casarin,A.MForati , E.Sote,B,Fazeka s.J. Tany and Zavezo,D.(2006) Evaluation of the efficacy of commercial hydrated sodium alumino silicate to reduce the toxicity of T-2 toxin in broiler chickens Poultry Scin.:201-209

Chowdhury, H.S.R. Smith ,T. K. Boermans ,H.J. and Wood ward ,B,(2005) : Effects of feed-Born fusarium mycotoxin on hematology and immunology of laying hens Poultry Science Vol. 84 :1841- 1850

Coffin,J.L. and Combs ,J.F.(1981): Impaired vitamin E status of chicken caused by T-2 .Poultry Scin.,54 :1043

Corrier, D.E.(1991): Mycotoxicosis Mechanism of immunosuppression Veterinary immunology and immune pathology. Vol.30 No 1 :73-87

Demello ,J . and Macdonald ,A.M.C. (1977): Mycotoxin Animal feed Science Technology

Dimic ,D. J.;Nesic ,K., and Sefer ,D.(2011): Mycotoxicosis of poultry caused by Trichothescenes Bio-technicology in animal husbandry 27(31) .:713-719

Dirish .C.K. and Levego W.Da G.(2004): Evaluation of modified Glucomanan (Mycosorb) and hydrated sodium calcium alumino silicate to ameliorate the individual and combined toxicity of Aflatoxin and T-2 toxin in broiler chicken Proceedings of Australian poultry science symposium Vol 16 :126-129 Sydney Australia

Ducan, D.R **(1955)** Multiple flats Biometrics 17 :31-34

Dumas, B.T and Biggs, H.G.(1972): Standard methods of clinical chemistry Ed Academic Press New York

Eriksen, G.S.(2003): Metabolism and toxicity of Trichothesins Doctoral Thesis Swedish Univ. of Agric .Sc .Uppsala

Forat, M. and Douglas Zaveso(2013): Low dosage efficacy of a commercial purified phylosis cato to reduce the toxicity of T-2 toxin in broiler poultry Scin(92)

Fosti , Pi Pricip, And Berti ,G. (1980) Urea of 3,5,2 hydroxyl benzene sulphoric acid / aminophenozon chromogenic syotinin direct enzymatic assay of an uric acid in serum and urine .Clin. Chem. 26 (**2**) :227-230

Garg, S.K.(2000) Veterinary Toxicology pp.224-228 CBS Publisher Distributors ,New Delhi

Gindler, E. M. and **King,J.D.(1972):** Rapid Colorimetric determination of calcium in biological fluid with methylene blue Am .j.Clin.Path.58:379-382.

Hassan, Z.U., Khan, M.Z. Khan , A. **.Joved**, I., **Hassan,Z**. **(2012**): Effect of individual and combined administration of Ochratoxin A and Aflatoxin B1 in tissues and eggs of white leghorn breeder hens . J. Sci. Food Agric.92 (7) :1540-1544

Heidence ,V.D. Bais ,Gerh Ardt ,Rosallisis(1994) Approved recommendation on IFCC Methods for the measurement of catalystic concentration of enzymes Part 8.IFCC method for LDH Eur ..j. Clin.Chemist. Clin.Bioch..,**32** .639-655

Henry, R.J. (1974): Clinical Chemistry Principles and techniques 2nd Ed. Her port and Row Hagerstown. M.D.862

Hoerr,F J ,Carlton W.W .Y . Agen,B. Joffe,A.Z.(1982) : Mycotoxicosis caused by either T-2 toxin or Diacitoxysinpemol in the broiler chickens Fund App; Toxicol 2:121-124

Hoerr ,F.J.(2003): " Mycotoxicosis " Diseases of poultry ,11th Ed. Iowa :110301132

Hu,X.I.uyi; Zhao ,Mouming and Huang ,Huihua (2010): Modification of pine apple peel fiber as metal ionic adsorbents through reaction with succinic anhydride in pyridia and Dimethyl sulphoxide .J. water Inviron . Res.**Vol.82 No.8 :**733-741\

Jain , H.C.(1986): Schlam s veterinary Hematology 4 th Ed .,Lea and Figer, Philadelphia, USA

Jordan, F.Pattison, M., Alexander, D. and Faragher, T.(2002): Poultry diseases 5Th Ed. Pp.397-401 Souder Comp. London

Kamal avenkatesh, P.(2003) : Individual and co pined effects of Cyclopiazonic acid and at-2 toxin in broiler chicken M. V .Sc. Submitted to tanilmands Veterinary and Animal Science University Chemmol India

Kaneko, J. J. ,Harvey ,J.W. and Bruss, M.T.(1997): Clinical ,Biochemistry of domestic animals 5th Ed. Academic Press California

Karaman, M. ;Basmacioglu ,H.; Ortatalli ,M.and Oguz ,H.(2005) Evaluation of the detoxifying effect of yeast Glucomanan on aflatoxicosis in broiler as assessed by growth examination and histopathology. Br. Poultry Sci. 46 ,:394-400

Krishnamoorthy, P. **Vairamuthu, S. Bolachandra, C. and Murolimanohar B.(2006**): Chlorpyriphos and T2 toxin induced Haemato0Biochemical alterations in broiler chicken International journal Poultry Science Vol 5 No 2:173-177.

Kubena ,L.F.,R., Harvey ,W. E ,Huff , M. H. Elmissald A. G. Yercin ,T.D. Philps and Rotting hous G.E.(1993): Efficacy of hydrated Sodium calcium alumino silicate to reduce the toxicity of Aflatoxin and diacetoxyseirp emol poultry Sci.,72 :51059

Kubena, L,F. Harvey ,R.B., Baily ,R.H.,Buckley ,S.A. and Rottinghous ,G.E.(1998): Effects of hydrated sodium calcium alumino silicate (T – bind) on Mycotoxicosis in young broiler chickens.

Maria Weidner, Sabiinse Huwel, Franzisk Ebesrt, Tanja Schwerdtle, Hans Joachim Galla,and Hans Ulrich Humpf(2013) Influence of T-2 and H T-2 toxin on the blood brain Perrier in vitro new experimental hints for neurotoxic effects J.pone. March 27

Marijana, Sokolovicv ,. Farag, V. Rhovac,V. Ramic,S. and Simprag ,P.(2007) : Chicken nucleated blood cells as a cellular model for gens toxicity testing using the b Commit Assay Food Chemical Toxicity, Vol.45 ,No 71:2165-2170

Marijana, Sokolovic, Nerica Garag Vrhoova and Borka Simpraga(2008): T -2 toxin incidence and toxicity to poultry Arh. Hig Rada Toksikol 59:43-52

Medina, J.CJ. Fierro, J., Lara.V. Brito , and Forat ,M.(2010) The effect of 1.2 ppb T-2 toxin on performance , lesions and general health of male broilers and the efficiency on organo silicate Poultry Scin. 89 Suppl:282-283

Meloche . J.L. and Smith ,T.K.(1955(: Altered tissue amino acid metabolism in acute T-2 toxicosis Proceeding of the Society for Experimental Biology and medicine Vol.210:260-265

Moursi , K.M.; Misa ,M. **Gharieb and Hala ,M. El-Genady (2008):** Pathological and immunological studies on dilatory T-2 toxin with concurrent *E. Coli* infection in chickens and some relevant control trials SCVMJ ,XIII (2) :565

Pand, V.V., Kerkore, N.V. and Bhamdarkar,|(2006): Effect of. **T-2 toxin** on growth performance and Haemato biochemical ultra ions .Indian J. of Exp. Biol. Vol. 44 January, 2006: 86-88

Parcell .S.(2002) : Smilfurin in human nutrition and applications in medicine Altern.Med.Rev. Feb., 7 (1):22-44

Pestka, J .J. Zhou ,H. R. Moony ,Y, Chug, Y.J. (2004) Cellular and molecular mechanism for immune modulation by deoxy - nivalenol and other Trichothecenes: Unraveling a paradox Toxicol. Lett 153: 61-73

Peters ,T.(1968) : Colorimetric method for determination of total serum proteins .Clin. Chem., **14**:1147-1153

Rajmon, R. ; Sedmikova, M. ,Jilak ,F.,Koubkova,M.,Hartlova,H,Barta,I.and Smerak,P.(2001): Combined effects of repeated low doses of aflatoxin and T-2 toxin on the Chinese hamster **.**Vet. Med.-Czech , 46 (11-12: 301-307)

Ramasamy,T.,**Varshneya ,C.and Katosh ,C.V.(2010**): Immune protective effect of sea buck thron (Hippophoe rhammoides) and Glucomanan on T-2 toxin induced immuono depression in poultry. Veterinary medicine vter. national Vol. 2010: 6 pages

Reitman , S. **and Frankel ,S.(1957):** Colorimetric method for determination of Serum Glutamic Oxalo acetic and Glutamic Pyruvic Transaminase Am.J.Path.,**26**:1-13

Rizzo, A., Atroshi .R., Hervi, T. and Saloviemi , H.(1992): The hemolytic activities of Deoxy -nivelenol and T-2 toxin Nat.Toxins.7:106-110

Rotter, B.A. Prelacy, D.B.and Pestka ,J.J.(1996) Toxicology of Deoxy nivalenol (Vemitoxin) J. Toxicol. Inviron .Health 48:1-34

SAS (2006): Statistical analysis system. User guide Statistics (SAS) Institute Carvorth Carolina USA

SCF, Scientific Committees on feed (2001): Pinion on the Fusarium toxins part 5 T-2 toxin and H T-2 toxin European commission health and consumer protection Directorate general SCF/CN/T O M M C 125 Evv. 6 Final

Schollenberger ,M., Muller ,H.M. ,Rufle ,M.,Suchy ,S.,Planke,S. and Drochner, W. (2006): Natural occurrence of 16 Fusarium toxins in grains and feed stuffs of plant origen from Germany Mycopathologia 161 ,43-52

Trinder, P.(1969) : Determination of Glucose in blood using glucose oxidase wth an alternative Oxygen acceptor Ana .Clin. L.I.N. Biochem **6** .24

Wahlefeld, A.W.(1974): In Methods of enzymatic analysis " Vol 5 Hu Bergemyer , Ed Academic Press New York : 1821-1835

Yohannes ,T. ,Sharma ,A.K.Singh ,D. Sumi ,V.(2013) :Experimental Haemato Biochemical alteration in broiler chickens fed with T-2 toxin and co-infected IB V open Journal of Veterinary Medicine Vol **3** No (5)

Yadav , S. S. ,Makhopadhayay ,S.K. and Purohit , K.(2003): Experimentally induced Chlorpyriphose toxicity in broilers Haemato Biochemical and patho.

morphological studies in 20 th Annual Conference of Indian Association of Veterinary Pathologist 31 :103

Xue,C.Y., Wang ,G.U, Chen,F.Zhang,X.B., Biy.Z. and Cao,.Y.C.(2010): Immuono pathological effects of Ochratoxin and T-2 toxin combination on broilers. J. Poultry Sci.Vol.89 Issue 6 :1162-1166