



EFFECT OF NORMAL AND SYNTHETIC ANTI MYCOTOXIN ON LEVELS OF NEUROTRANSMITTER HORMONES AND IMMUNE STATE IN BROILER CHICKENS

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Abstract

Mycotoxins one of major problems in poultry industry had wide different of harmful impact to birds. The study investigate the role of natural (Bentonite) and synthetic (Mycofix® Plus) antimycotoxin substance on levels of neurotransmitter hormones (Dopamine and Serotonin) and immune state (CD4, CD8) levels with contaminated diet with aflatoxin in broiler, A total of 180 broiler chicks were divided randomly into six groups T1-Control +ve chick's given contaminated diet by mycotoxins only. T2-chick's given Bentonite at a dose (5 kg / ton of feed) with contaminated diet by mycotoxins. T3-chick's given Mycofix® Plus at a dose (1.5 kg / ton of feed) with contaminated diet by mycotoxins. T4-Active control chick's given Bentonite® at a dose (5 kg / ton of feed) with not contaminated diet (free mycotoxins). T5-chick's given Mycofix® Plus at a dose (1.5 kg / ton of feed) with not contaminated diet (free mycotoxins). T6-chick's given not contaminated diet only (free mycotoxins). The result of Dopamine was significantly ($P<0.05$) higher in (T5) and no significant difference at ($P<0.05$) in (T2,T3,T6) respectively, then at T1 finally, T4 had less significant difference at ($P<0.05$) at 40 days old. The results of serotonin in 20 and 40 days old were recorded increased with significant difference at ($P<0.05$) in T5 then no significant difference at ($P<0.05$) between (T2,T3) respectively, followed (T4,T6) respectively. Finally T1 had less value. Results of CD4 in 20 and 40 days old were recorded increased with significant difference at ($P<0.05$) in (T4,T5) respectively and no significant difference between (T2,T3,T6) respectively. Then T1 had less value with significant difference at ($P<0.05$). The results of CD8 in 20 and 40 days old was recorded increased with significant difference at ($P<0.05$) in T5 among all groups. There were no significant difference at ($P<0.05$) between (T4,T6) respectively, then also between (T2,T3) respectively. Finally T1 had less value with significant difference at ($P<0.05$). In Conclusion mycotoxin had harmful impact on different biological and immunological parameters. Natural and synthetic anti-mycotoxin reduce mycotoxin levels in diet within the time. Natural and synthetic anti-mycotoxin maintains the immunological response by improved (CD4,CD8) levels, as well as improved levels of dopamine and serotonin (neurotransmitter hormones).

Key words: mycotoxins, bentonite , Mycofix® Plus, Dopamine, serotonin, CD4, CD8.

Introduction

Mycotoxins are product of metabolites produced of fungi that can cause severe harmful effects to poultry (Zain, 2011; Amjed, 2018). Aflatoxins caused major problems in the poultry with extensive economic losses. (Amjed, 2017). The high levels of mycotoxins in diet can caused acute mycotoxicoses and high mortality rate, while low levels caused chronic mycotoxicoses with or without clinical signs and had immunosuppressive effects (Cheng *et al.*, 2001).

Bentonite is natural formed by highly colloidal and plastic clays mainly composed from o montmorillonite

(Bergaya and Lagaly, 2013). Bentonite is generated from the alteration of volcanic ash, and predominantly of smectite minerals (montmorillonite) (Boylu *et al.*, 2010). And the processing anti-mycotoxins Mycofix® Plus represent especial developed factor feed supplements for animal protection and decreased mycotoxins concentration in contaminated diet and is proper for use in poultry, pig and ruminant diet as well as fish and shrimp feed (www.biomin.at, www.biomin.net.)

Materials and Methods

Chicks of experiment and Experiment Location

Hundred eighty broiler chicks (Ross 308) (Turkey

source) mixed sexes. At one day old were used in our experiment. Chicks were bred in the poultry field / Department of Pathology-College of Veterinary Medicine -University of Baghdad/Iraq for 40 days from 10 January to 20 February 2019.

Diets composition and contents

All birds in this study were offered feed and water *ad libitum*. Mycotoxin levels in diet were detected every ten days by (ELISA) for (aflatoxin, ochratoxin, T2 toxin) for all groups (Amjed, 2019), as following:

- Experimental Design:

T1-(30) chicks were Fed on contaminated diet only by mycotoxins.

T2-(30) chicks were Fed on Bentonite® at a dose (5 kg / ton of feed) with contaminated diet by mycotoxins.

T3-(30) chicks were Fed on Mycofix® Plus at a dose (1.5 kg / ton of feed) with contaminated diet by mycotoxins.

T4-(30) chicks were Fed on Bentonite® at a dose (5 kg / ton of feed) with not contaminated diet.

T5-(30) chicks were Fed on Mycofix® Plus at a dose (1.5 kg / ton of feed) with not contaminated diet.

T6-(30) chicks were Fed on not contaminated diet only.

Parameters that have been studied

A. Nervous transport hormones concentration:

1. Serotonin: According to protocol of company Bioassay Technology Laboratory. Chicken Serotonin ELISA kit, Cat.No E0177Ch.

Standard Curve Range: 0.5ng/ml - 150ng/ml

Sensitivity: 0.28ng/ml

2. Dopamine: according to protocol of company Bioassay Technology Laboratory. Chicken Dopamine ELISA Kit, Cat.No E0275Ch.

Standard Curve Range:0.05ng/ml-10ng/ml

Sensitivity: 0.018ng/ml.

B. General immunological status CD4+ and CD8+

1. CD4+ : According to protocol of company Bioassay Technology Laboratory. Chicken Cluster of Differentiation 4 ELISA Kit (CD4). Cat. No E0001Ch.

Standard Curve Range: 20ng/L - 7000ng/L

Sensitivity: 10.25ng/L.

2. CD8+: According to protocol of company Bioassay Technology Laboratory. Chicken Cluster of Differentiation 8 ELISA Kit (CD8). Cat. No E0002Ch.

Standard Curve Range: 30ng/L - 10000ng/L

Sensitivity: 15.75ng/L.

Statistical Analysis

This program (Statistical Analysis System- SAS - 2012) to detect the study difference factors in research parameters. Least significant difference-LSD test was used to significant compare between means in this search (Sas, 2012).

Results and Discussion:

Dopamine

The results of dopamine in 20 days old showed no significant difference at (P<0.05) among all groups. In 40 days old were recorded increased with significant difference at (P<0.05) in (T5) (6.646) then among other groups and no significant difference at (P<0.05) in (T2,T3,T6) (6.083-6.153-6.138) respectively, then at T1 (5.594) finally, T4 (5.257) had less significant difference at (P<0.05).

The results between two aged (20-40) when compared showed no significant difference at (P<0.05) among all groups except T4 (0.559) showed significant difference at (P<0.05) between them.

In T5 had higher levels of dopamine that agree with (Edens, 1987) were also found higher levels of dopamine in the brain of chicken with normal behavior due to good programmer feed increased dopamine levels when present in the some brain parts in both humans and poultry following good action (Van Erp and Miczek, 2007). The addition of Mycofix Plus® to diet significantly increased dopamine because was additive with other properties **Table 1:** Mycotxine percentage (ppb- part per billion) in different (Groups & Age).

T6 ppb	T5 ppb	T4 ppb	T3 ppb	T2 ppb	T1 ppb	Day old	Feed type
A:0.9	A:0.0	A: 0.0	A:22	A:22	A:22	1	Mixed
O:0.0	O:0.0	O:0.0	O:1.2	O:1.2	O:1.2		
T2:0.0	T2:0.0	T2:0.0	T2:117	T2:117	T2:117		
A:0.9	A:0.0	A:0.0	A:19	A:22	A:22	10	Mixed
O:0.0	O:0.0	O:0.0	O:0.8	O:1.2	O:1.2		
T2:0.0	T2:0.0	T2:0.0	T2:88	T2:101	T2:117		
A:1.7	A:0.0	A:0.0	A:9.8	A:16	A:22	20	Mixed
O:0.0	O:0.0	O:0.0	O:0.0	O:0.0	O:1.3		
T2:0.0	T2:0.0	T2:0.0	T2:22	T2:60	T2:119		
A:2.0	A:0.0	A:0.0	A:6.4	A:11.7	A:22	30	Mixed
O:0.0	O:0.0	O:0.0	O:0.0	O:0.0	O:1.2		
T2:0.0	T2:0.0	T2:0.0	T2:0.0	T2:0.0	T2:118		
A:2.6	A:0.0	A:0.0	A:5.8	A:8.4	A:24	40	Mixed
O:0.0	O:0.0	O:0.0	O:0.0	O:0.0	O:1.0		
T2:0.0	T2:0.0	T2:0.0	T2:0.0	T2:0.0	T2:115		
The permitted dose: { Afla toxin – 20ppb (A) } / { Ochra toxin-5ppb (O) } / { Trichothecene toxin-150 ppb (T2) }							

Table 2: Elisa kit for chicken mycotoxin.

Kits	Origin
Mycotoxins	Synbiotics-USA
CD4	Shanghai-China
CD8	Shanghai-China
Dopamine	Shanghai-China
Serotonin	Shanghai-China

which was improved by the addition of enzymes (depoxydase and lactonase) in order to reduce mycotoxins (Fuchs *et al.*, 2002). Mycofix plus® was effectively used in poultry for amelioration of aflatoxin and ochratoxicosis (Garcia *et al.*, 2003), then among other groups no significant difference at (P<0.05) (T2,T3,T6) respectively, then (T1 and T4) had less significant difference, the toxins caused depletion or decreased in dopamine concentration and ratio in the central nervous system have been involved in imbalance behaviors, including harm impact to human and animals (Unis *et al.*, 1997) and savagery in birds (Barofsky *et al.*, 1983). And Which fed with not contaminated food and bentonite, can restrict the mycotoxins during ingestion and decreased or eliminate levels of toxicity (Dos Anjos *et al.*, 2015). Bentonite reduces the bioavailability of mycotoxins (Robinson *et al.*, 2012) and decreases the cocentration of (Aflatoxin M1) product of a hydroxylated metabolism of aflatoxin (Mitchell *et al.*, 2013). While some studies investigate the dopamine concentration increased in the brain and other parts of body due to mycotoxicosis, Eisenhofer *et al.*, (2004).

Result of Serotonin

The results of serotonin in 20 days old were recorded increased with significant difference at (P<0.05) in T5 (76.782) then no significant difference at (P<0.05) between (T2,T3) (68.992-67.657) respectively, followed (T4,T6) (72.086-73.039) respectively. Finally T1 (54.860) had less value. In 40 days old were recorded increased with significant difference at (P<0.05) in T5 (78.912) then followed by T4 (73.070), T6 (70.337), T3 (67.221) and T2 (65.210) Finally, T1 (52.832) had less significant

Table 3: Effect of Dopamine concentration at 20-40 days old.

T	DOP (ng/ml): B-P/20	DOP (ng/ml): B-P/40	LSD value
T1	5.893 a	5.594 bc	0.394 NS
T2	6.142 a	6.083 ab	0.447 NS
T3	6.129 a	6.153 ab	0.361 NS
T4	6.209 a	5.257 c	0.559 *
T5	6.379 a	6.646 a	0.382 NS
T6	6.226 a	6.138 ab	0.298 NS
LSD value	0.622 NS	0.681 *	-
Means having with the different letters in same column differed significantly. * (P<0.05).			

difference at (P<0.05).

The results of Serotonin compared between two aged (20-40) showed no significant difference at (P<0.05) among all groups.

In T5 increased and unchanged blood 5-HT concentrations have been found in association with normal behavioral functions, including good programmer feed (Moffitt *et al.*, 1998). Secretion of blood Serotonin associated with specific good behavioral guideline (Unis *et al.*, 1997). The addition of Mycofix Plus® to diet significantly increased of serotonin because improvement of daily mean weight gain and daily mean feed intake (Lindemann *et al.*, 1993). Mycofix plus® was effectively used in poultry for reduce of aflatoxin levels (Garcia *et al.*, 2003). In T1 reduction of serotonin levels and ratio in the some part of brain (CNS) caused abnormal behaviors, and caused harm impact to human and animals (Felver-Gant, 2011) and cannibalism in birds and toxicity. (Barofsky *et al.*, 1983). In toxicologic research, FA was shown to low levels of pineal 5HT, 5-hydroxyindoleacetic acid (5HIAA) and 5-hydroxytryptophol (5HTOL) (Porter *et al.*, 1995). In other reported by Swamy *et al.*, (2004), high elevate in cortex serotonin concentration in broiler chickens as a result of bad feeding program and mycotoxicosis.

Result of Immunological parameter

- Result of Cluster of Differentiation 4(CD4).

The results of CD4 in 20 days old were recorded increased with significant difference at (P<0.05) in (T4,T5) (3822.826-3857.909) respectively and no significant difference at (P<0.05) between (T2,T3,T6) (2937.137-3078.303-3237.528) respectively. Then T1 (2001.235) had decrease value with significant difference at (P<0.05). In 40 days old were recorded increased with significant difference at (P<0.05) in (T4,T5) (4016.481-4088.921) respectively, then followed by T6 (3673.533), T3 (3140.590) and T2 (2830.702) respectively, Finally

Table 4: Effect of serotonin concentration at 20-40 days old.

T	SERO (ng/ml): B-P/20	SERO (ng/ml): B-P/40	LSD value
T1	54.860 c	52.832 d	4.38 NS
T2	68.992 b	65.210 cd	3.97 NS
T3	67.657 b	67.221 bc	3.06 NS
T4	72.086 ab	73.070 ab	5.12 NS
T5	76.782 a	78.912 a	3.94 NS
T6	73.039 ab	70.337 b	3.79 NS
LSD value	8.02 *	7.94 *	-
The different letters in same column means differed significantly. * (P<0.05).			

Table 5: Effect of CD4 concentration at 20-40 days old.

T	CD4 (ng/l): B-P/20	CD4 (ng/l): B-P/40	LSD value
T1	2001.235 c	1881.690 d	347.24 NS
T2	2937.137 b	2830.702 c	296.56 NS
T3	3078.303 b	3140.590 bc	302.42 NS
T4	3822.826 a	4016.481 a	475.91 NS
T5	3857.909 a	4088.921 a	362.67 NS
T6	3237.528 b	3673.533 ab	452.80 NS
LSD value	551.94 *	647.07 *	-
The different letters in same column means differed significantly. * (P<0.05).			

T1 (1881.690) had less value with significant difference at (P<0.05).

The results of CD4 when compared between two aged (20-40) showed no significant difference at (P<0.05) among all groups.

The results showed that feed supplementation with bentonite in T4 activate the T-lymphocyte proliferation process, particularly of (CD4 + T cells) and increase in the percentage of (CD4/T-cells) in the experimental groups due to activation by Bentonite as an (antigen) (Ozesmi *et al.*, 1986). Our study also indicate an increase in the (CD4/ T-cell) ratio these results to bentonite confers a activate on immune function in broiler chicken and enhance the resistance of broiler chicken against infection (Tarpey *et al.*, 2007). In T5 The addition of Mycofix Plus to diet significantly increase of T-lymphocyte and splenic plasma cell number, Mycofix Plus effect on mycotoxins by breaking particular function of toxin (Çelik *et al.*, 2000). Then T1 had decrease value with significant difference at (P<0.05) according to Chen *et al.*, (2014) was observed reduction of CD4+ T-cells in the broilers infected by aflatoxin. Previous other studies have investigated that aflatoxin has immunosuppressive impact to general (humoral and cellular) immunity (Dugyala *et al.*, 1996). In chick, aflatoxicosis decrease the percentages of (CD4+/ T-cells) in the thymus and spleen (Chen *et al.*, 2014). This is similar study report by Kamalavenkatesh *et al.*, (2005), decrease the thymus and spleen (CD4+ T-cell) in broilers fed diets containing mycotoxin.

- Result of Cluster of Differentiation 8(CD8).

The results of CD8 in 20 days old was recorded increased with significant difference at (P<0.05) in T5 (5762.942) among all groups. There were no significant difference at (P<0.05) between (T4,T6) (5234.254-5397.731) respectively, then also between (T2,T3) (4960.338-5184.368) respectively. Finally T1 (4356.175) had less value with significant difference at (P<0.05). In 40 days old was recorded increased with significant

Table 6: Effect of CD8 concentration at 20-40 days old.

T	CD4 (ng/l): B-P/20	CD4 (ng/l): B-P/40	LSD value
T1	4356.175 c	3401.963 e	628.09 *
T2	4960.338 b	4678.264 d	455.48 NS
T3	5184.368 b	5643.452 bc	602.73 NS
T4	5234.254 ab	5936.026 ab	533.92 *
T5	5762.942 a	6269.384 a	593.02 NS
T6	5397.731 ab	5267.287 c	378.91 NS
LSD value	528.82 *	576.14 *	-
The different letters in same column means differed significantly. * (P<0.05).			

difference at (P<0.05) at (T5) (6269.384) among all groups. Then following by T4 (5643.452), T3 (5643.452), T6 (5267.287) and T2 (4678.264). Finally T1 (3401.963) had less value with significant difference at (P<0.05).

The results of CD8 when compared between two aged (20-40) showed significant difference at (P<0.05) at (T1,T4) respectively, but had no significant difference at (P<0.05) among (T2,T3,T5,T6) respectively.

In T5 the addition of Mycofix Plus to feed diet significant improve T-lymphocyte and splenic plasma cell count, Mycofix Plus has been capable to reduce the mycotoxins by breaking particles (Çelik *et al.*, 2000). addition of mycofix plus to the diet can elvate caused the number of (CD8/ T-cell) in the broilers and this increase in the percentage of CD8 ratio, mycofix can modulate activity of CD8 (Colic and Pavelic 2000). In T1 had less value with significant difference at (P<0.05) according to Chen *et al.*, (2014) observed reduced percentages of CD8+ T-cells in the broilers administered aflatoxin. Previous other studies have investigated that aflatoxin has immunosuppressive impact to general (humoral and cellular) immune response (Dugyala *et al.*, 1996). In chick, aflatoxicosis decrease the percentages of (CD8+/ T-cells) in the thymus and spleen (Chen *et al.*, 2014). Aflatoxicosis reduce the immune response in poultry, as well as can prevent the development of the (thymus gland and bursa of Fabricius) and causes impair at both cellular and humoral immune response (Celik *et al.*, 2000)

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