

## INFLUENCE OF MYCOTOXINS ON IMMUNE RESPONSES AGAINST SALMONELLA TYPHIMURIUM INFECTION OF BROILER CHICKENS

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### ABSTRACT

Forty broiler chickens, One - day old were randomly divided into four equal groups: 1<sup>st</sup> group was immunized with 0.5 ml of whole sonicated salmonella antigens (WSSAgS), protein concentration 1.89 mg/ml. Two dose two weeks intervals, S/C at 7 days old and the chicks fed contaminated diet with mycotoxins for 7 week, 2<sup>nd</sup> group was immunized with WSSAgS only and treated as 1<sup>st</sup> group, 3<sup>rd</sup> group fed diet contaminated with mycotoxins and 4<sup>th</sup> group was fed normal diets and served as control negative group, At 30 days, skin test, phagocytic index and serum levels of antibody titers were done, then 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> groups were inoculated with high dose of virulent *S.typhimurium* , (1ml containing  $1 \times 10^{12}$  CFU/ml ), I/V, and 4<sup>th</sup> group was inoculated I/V, 1ml sterile normal saline and served as control negative group , all chicks were sacrificed at 3 weeks post infection, it was recorded that mycotoxin suppress the cellular and humoral immune responses , phagocytic activity , in addition to high mortality rate were found in chicks fed contaminated diet with and without immunization.

**Keywords:** mycotoxins, *Salmonella typhimurium*, WSSAgS, antigens, serum

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تأثير السموم الفطرية على الاستجابات المناعية ضد عدوى السالمونيلا تايفيموريوم للدجاج اللامح

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الاستاذ

الباحث

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المستخلص

تم اخذ اربعين من دجاج اللحم بعمر يوم واحد والتي تم تقسيمها وبصورة عشوائية الى اربعة مجاميع متساوية وكان العلاج على الشكل التالي: المجموعة الاولى فقد تم تحصينها ب 0.5 مل من WSSAgS وبتركيز بروتيني 1.89 ملغم/ مل وبجرعتين ويفاصل زمني وهو اسبوعين و S/C للفرايج بعمر 7 ايام وفي نفس الوقت قد تم اطعامها بغذاء ممزوج بالميكوتوكسين. ولمدة 7 اسابيع اما المجموعة الثانية فقد تم تحصينها بالمستضد WSSAgS فقط كالمجموعة الاولى. والمجموعة الثالثة فقد اطعمت بغذاء ممزوج بمادة المايكوتوكسين فقط كالمجموعة الاولى. والمجموعة الرابعة فقد تركت بدون معاملة بعليقة طبيعية وعملت كمجموعة سيطرة سالبة. وبعد 30 يوما من عملية التطعيم تم اجراء اختبار الجلد ومستوى المصل للجسام المضادة بالاضافة الى تحديد النشاط البلعمي. ومن ثم المجموعة الاولى، الثانية والثالثة تحددت I/V بالجرعة العالية 1 مل الذي يحتوي على  $1 \times 10^{12}$  CFU/ml من السالمونيلا التايفيموريوم الضارية اما المجموعة الرابعة فقد تركت بدون اصابة مع حقنها بالوريد ب 1 مل من محلول الملح المعقم على انها مجموعة سيطرة للمجموعات الثلاثة السابقة، كما وان كل الفرايج قد تم ذبحها عند ثلاث اسابيع بعد الاصابة، نتائج الدراسة الحالية، سجلت بان المايكوتوكسين يكبح كل من الاستجابة المناعية الخلوية والخلطية والنشاط البلعمي وان معدل الوفيات العالي وجد لدى الفرايج المحصنة والغير محصنة التي اطعمت بغذاء ملوث وعليه يستنتج على ان الميكوتوكسين يستطيع اخماد الاستجابة المناعة ويزيد من حساسية الفرايج للاصابة بسالمونيلا التايفيموريوم .

الكلمات المفتاحية: دجاج اللحم، اختبار الجلد، النشاط البلعمي، مستضدات السالمونيلا.

البحث مستل من اطروحة الدكتوراه الباحث الاول

## INTRODUCTION

*Salmonella* is considered important public health and economic problems in human and animal's worldwide (16). Infection by this pathogen causes 1 million illnesses and 380 deaths every year in USA with economic losses excess of \$2.7 billion annually particularly *S.typhimurium* infection (8), Also in 2013, the United States Department of Agriculture Food Safety and Inspection Service reported 18% prevalence of *Salmonella* contamination in ground chicken (29). The immune response against *Salmonella* infection is very complicated and required interaction of innate and acquired immune responses (1), which are not completely understood and they required further researches, Poultry are commonly exposed to chronic form of mycotoxins due to intake of low concentration of these toxin for long period and they express chronic symptoms of mycotoxins such as poor growth, poor feed efficiency, decline in their production but acute mycotoxicosis may occur in poultry post-ingestion high concentration of the toxin that lead to acute clinical symptoms due to toxic effects on their vital organs ,immune response and other their physiological features in addition to high mortality (9). However, impair immune responses were recorded in human and animals consumption food contaminated with mycotoxins resulting in increase susceptibility of the host to infectious pathogens such as *Salmonella spp.* in addition the toxins themselves can change the virulence factors of the pathogens that associated with increasing microbial toxicity and its invasiveness ability to varies host tissue include immune cells (4). The mycotoxin can cause immunotoxic in the birds associated with suppresses cell mediated immunity CMI, humoral immunity and inflammatory response (5), there is few researches about the immunotoxic effects of mycotoxins in broiler therefore the aim of the present study was to determine the influence of mycotoxin on immune response against infection by *S.typhimurium* in broiler chickens.

## MATERIALS AND METHODS

A total of forty broiler chicks both (male and female), 1 day of age. were obtained from

a local commercial hatchery and then wing-banded and raised in a floor pens with clean wood shaving in an environmentally controlled electrically – heated room (the room temperature was maintained at 32 °C from d 1 to 7 (with 35-45% relative humidity) and then gradually reduced 2 °C per week. All chicks were vaccinated with Newcastle disease (ND) via drinking water at day 7 followed by booster dose of ND virus vaccine at day 17 and with Gomboro vaccine at day 14. Experimental period extended to 8 weeks.

**Mycotoxin analysis (AFB1, OTA, T-2).** All samples were homogenized to obtained 10 commercially poultry diet and a total 10 representative samples (1 kg per sample) were analysis for mycotoxin by ELISA assay kits in veterinary laboratories of general authority of veterinary medicine in Al-Nahta region. The detection limit for these mycotoxins was (10 ppb of AFB1), (1.4 ppb of Ochratoxin A), and (129.9 ppb of T-2 toxin).

### Bacterial strain

The strain of *S.typhimurium* was isolated from naturally infected chickens diagnosed by routine bacterial methods according to (22) and activated according to (7).

**Whole sonicated *S.typhimurium* antigens** prepared according to (26).

**Activity of phagocytic cells** of immunized groups and control group has been done according to the method of (15).

**Experimental Design:** Forty broilers (Rose), one day old, both sex, were randomly divided into four equal groups and treated as the following

-First group was fed diet contaminate with mycotoxin at age 7 days for 7 weeks and at 2 weeks age, it was immunized S/C with 0.5 ml of whole sonicated *S.typhimurium* antigens (WSS Ags) (protein conc. 1.89 mg/ml), two does, two weeks intervals.

-Second group was immunized with (WSSAgs) only treated as in 1<sup>st</sup> group with normal diet.

-Third group was fed contaminated food with mycotoxins as 1<sup>st</sup> group

-Fourth group was served as control negative group.

At 4 weeks post immunization, skin test was done in the immunized groups in all groups and blood samples were collected in

nonheparinized tubes by brachial vein puncture to determine the level of IgG in serum, as well as phagocytic activity, then 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> groups were inoculated with high dose of bacterial suspension of *Salmonella typhimurium* containing ( $1 \times 10^{12}$ ) CFU/ml (1 ml/ I.V) in wing vein Clinical symptoms and mortality rates were determine during the experiment course, and then all animals were sacrificed at 3 weeks post infection. Gross examination of internal organs was done, and small pieces of heart, spleen, liver, kidney, lung , bone , bursa and thigh muscle were fixed in 10% neutral buffer formalin for histopathological examination

## RESULTS AND DISCUSSION

**Delayed type hypersensitivity (DTH):** At 24hr post examination, the results of skin test showed that the mean skin thickness in the 1<sup>st</sup> and 2<sup>nd</sup> were 2.32 and  $3.06 \pm 0.05$ , respectively and these values were decrease at 48hr post examination as following:  $1.58 \pm 0.11$  and  $1.85 \pm 0.25$  in the 1<sup>st</sup> and 2<sup>nd</sup> groups respectively (Table: 1) There were significant differences ( $P < 0.05$ ) between the mean values of two groups after 24 hr post-examination, in which G2 ( $3.06 \pm 0.05$ ) showed the highest mean value

**Table 1. The mean values of the skin thickness of chicken after 30 day post immunization.**

Group	24hr	48hr
G1	A2.32b	B1.58±0.11ab
G2	A3.06±0.05a	B1.85±0.25a
LSD		

Means with a different small letter in the same column significantly different ( $P < 0.05$ ), Means with a different capital letter in the same row significantly different ( $P < 0.05$ )

**Serum antibodies (IgG):** The mean values of antibody titer (IgG) in 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> were  $5.31 \pm 0.54$ ,  $8.95 \pm 1.92$ ,  $4.99 \pm 0.27$  and,  $4.13 \pm 0.22b$ , respectively, Table 2.

**Table 2. Mean values and SE of serum levels of IgG Conc. (ng/ml) at 30 days post immunization.**

Groups	Mean±SE
G1	B5.31±0.54b
G2	A8.95±1.92a
G3	A4.99±0.27c
G4	4.13±0.22b
LSD	

Means with a different small letter in the same column significantly different ( $P < 0.05$ ), means with a different capital letter in the same row significantly different ( $P < 0.05$ ).

## Phagocytic activity

The results of statistical estimation of the ability of engulfing cells of killed microorganism were revealed mean values of phagocytic index of immunized group as (table: 3), in 1<sup>st</sup> and 2<sup>nd</sup> were  $0.41 \pm 0.03$  and  $0.79 \pm 0.02$  respectively, it showed increase significantly at level ( $P < 0.05$ ) in G2 ( $0.79 \pm 0.02$ ) compare to groups G1 ( $0.41 \pm 0.03$ ).

**Table 3. Mean values of Phagocytic activity index in immunization groups.**

Group	Mean±SE
G1	0.41±0.03b
G2	0.79±0.02a
LSD	

Means with a different letter significantly different ( $P \leq 0.05$ )

## Clinical symptoms and mortality

The main clinical symptoms expressed by life chickens, post infection with high dose in 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> group , were white diarrhea, decrease feed intake, increase water intake and lowered grwth rate. In addition to depression, dull feathers (loss of shining, paralysis of the legs (Fig:1) associated with inability to eat and drink as well as exited yellow liquids from mouth of the some infected birds, nervous cramps, whistling, and high mortality rates in broiler fed contaminated diets with and without immunization, these symptoms were moderated in the life immunized animals with moderate mortality (Table :4).



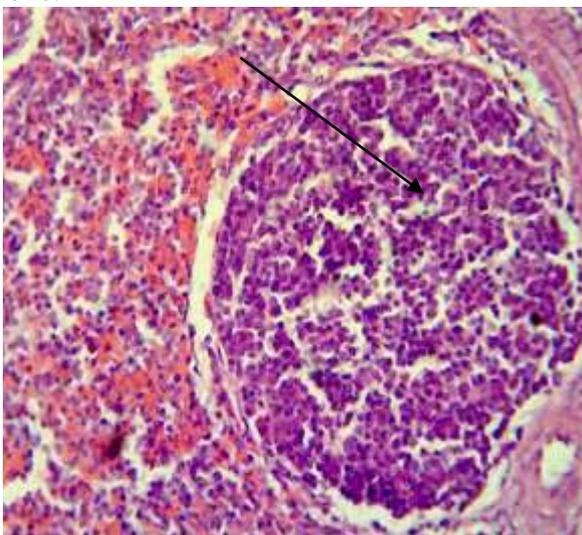
**Fig. 1. Bird in 1<sup>st</sup> group (1 week post infection) showed paralysis of the legs and unable of bird to walk**

**Table 4. Survival rate and mortality in all groups post infection with *S. Typhimurium***

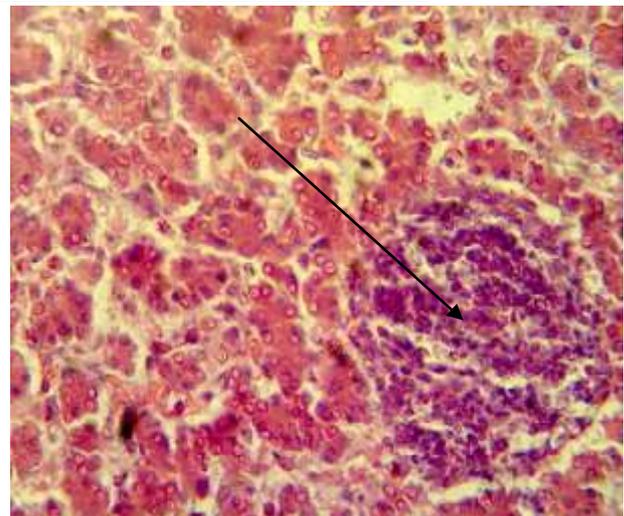
GROUP	NO OF BIRDS	DEAD BIRDS	SURVIV E BIRDS	% OF SURVIVAL ANIMALS	% OF MORTALITY
G1	10	8	2	20	80
G2	10	4	6	60	40
G3	10	5	5	50	50
CHI SQUARE VALUE					
P	0.04				

**Histopathological results of infected groups**

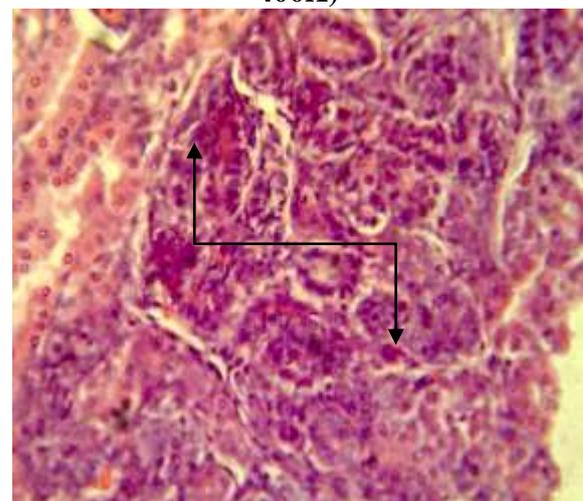
**G1 (Immunized with Mycotoxin):** The spleen showed depletion of white pulp with hemorrhage and congested red pulp (Fig.:2) the liver revealed granulomatous lesion in their parenchyma, with dilated sinusoids (Fig.:3) and fibrin networks deposition, Also there was edema between cardiac muscle fiber, while section of the lung were showed fibrin deposition in the interstitial tissue with congested blood vessels, increase thickness of the epithelial layer of parabronchi , heterophils infiltration, and edema in the wall of the bronchi. The lesion in the kidney characterized by presence of mononuclear cells aggregation in the interstitial tissue and severe congestion of blood vessels (Fig.:4).with no clear lesion in bone.



**Fig. 2. Section in the spleen of animal in (G1) treatment with mycotoxin and immunization with WSSAgS and infection with *S.typhimurium* at 2 weeks post infection shows moderate hyperplasia of white pulp (arrow) (H &E stain 400X)**



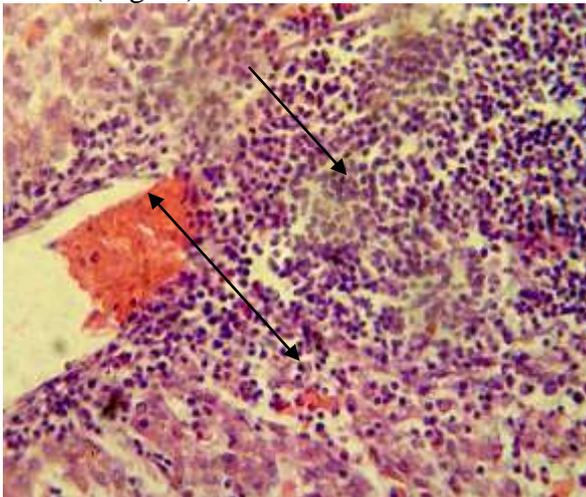
**Fig. 3. Section in the liver of animal in G1 treatment with mycotoxin and immunization with WSSAgS and infection with *S.typhimurium* at 3 weeks post infection shows granulomatous lesion in the liver parenchyma (arrow) (H &E stain 400X)**



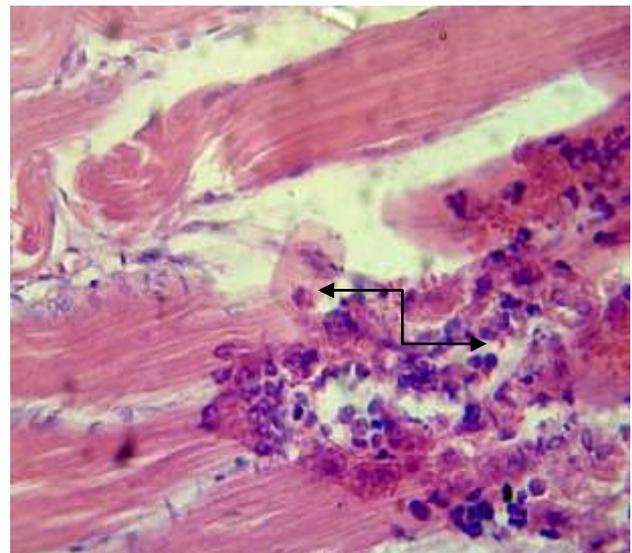
**Fig.: 4. Section in the kidney of animal treatment with mycotoxin and immunization with WSSAgS at 3 weeks post infection with *S.typhimurium* shows mononuclear cells aggregation in the interstitial tissue (arrow) (H &E stain 400X)**

### G2 (Immunized only without Mycotoxin group)

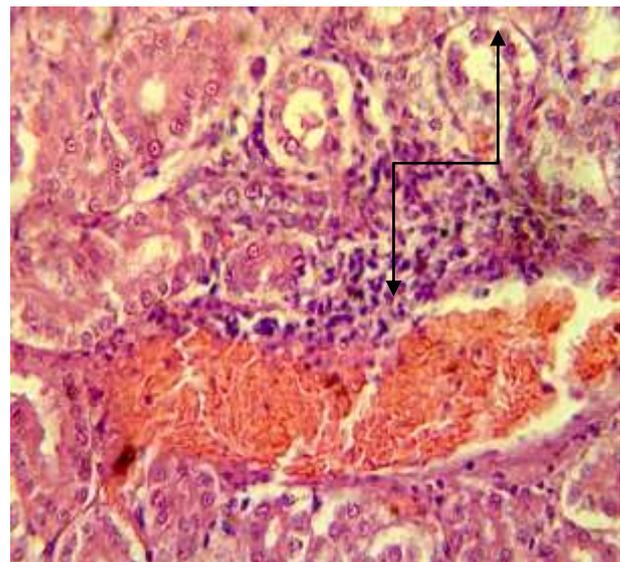
Histopathological changes were observed in liver characterized by presence of moderate to marked mononuclear cells aggregation around congested blood vessels with fibrin deposition in parenchyma of liver and dilated sinusoids (Fig.:5), Also inflammatory cells particularly heterophils infiltration between fragment fibers of skeletal muscle (Fig.:6), but there was no clear lesion in heart and in bones. In addition to above changes, sections from kidneys also were showed hemorrhage in the interstitial tissue, mononuclear cells infiltration between glomeruli, mononuclear cells aggregation in the wall of congested blood vessels with acute cellular degeneration of tubular epithelial lining (Fig.:7). Also infiltrated of a few of MNCs in the wall of the bronchiole of the birds lungs. The spleen showed amyloid like substance deposition around white pulp. While the bursa of Fabricius showed focal necrosis of lymphoid follicle (Fig.: 8).



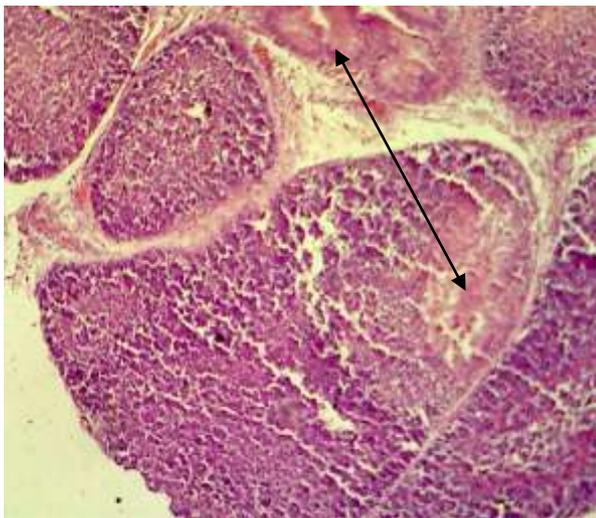
**Fig.: 5.** Section in the liver of animal in G2, immunization with WSSAg, at 1 week post infection with *S.typhimurium* shows congested blood vessels (arrow), inflammatory cells infiltration (H &E stain 400)



**Fig.: 6.** Section in the skeletal muscle of animal in G2, immunization with WSSAg, at 2 weeks post infection with *S.typhimurium* shows heterophils infiltration between fragment muscle fiber (arrow) (H &E stain 400X)

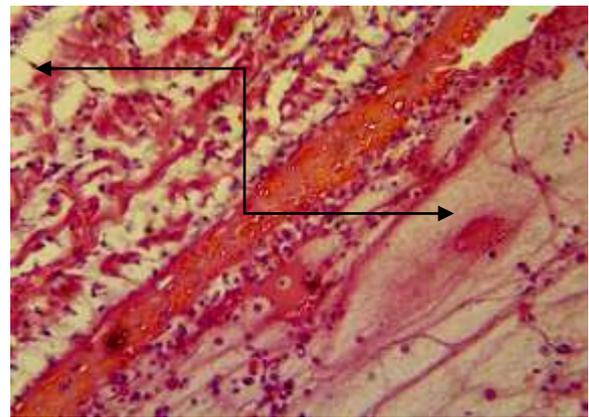


**Fig.: 7.** Section in the kidney of animal in G2, immunization with WSSAg, at 3 weeks post infection with *S.typhimurium* shows mononuclear cells aggregation in the wall of congested blood vessels with acute cellular degeneration of epithelial (arrow) (H &E stain 400X)

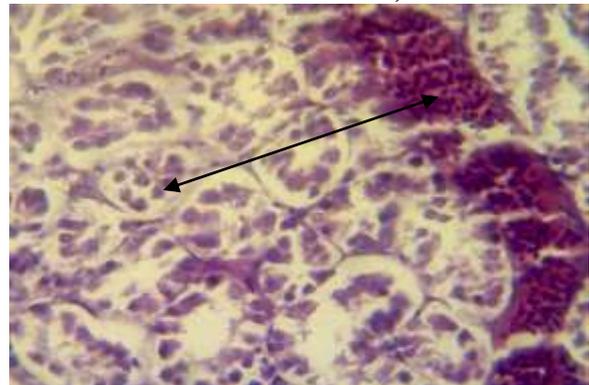


**Fig. 8.** Section in the bursa of animal in G2, immunization with WSSAg, at 1 week post infection with *S.typhimurium* shows focal necrosis of lymphoid follicle (arrow) (H &E stain 100X)

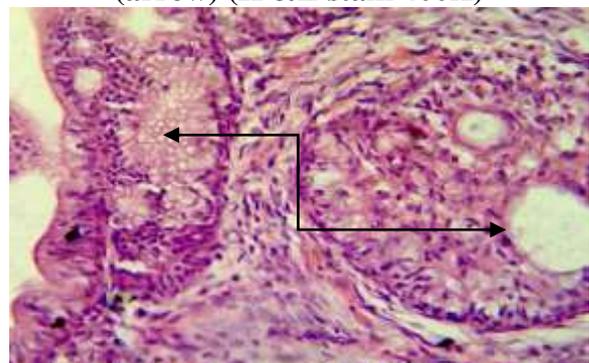
**G3 (Nonimmunized with mycotoxin group).** Animals fed contaminated diet and infection with *S.typhimurium*. The heart shows fibrin deposition, edema and inflammatory cells infiltration in the pericardium in addition to inflammatory cells infiltration between cardiac muscle fiber (Fig.: 9), while in kidney shows severe hemorrhage in the interstitial tissue with acute cellular degeneration (Fig.:10), also severe hemorrhage in the capsular region with severe vacuolar degeneration of epithelial cells may noticed in other animals , While the lesion of bursa revealed severe depletion of lymphoid follicles with cystic formation (Fig.:11), The spleen shows severe depletion of white pulp with bacterial colonies in spleen (Fig.:12), the main lesion of the liver in bird of this group consisting of severe destruction of hepatocytes with large necrotic area accompanied with granulomatous lesion ,in another chicken severe portal fibrosis of liver parenchyma (Fig.:13), and skeletal muscle showed inflammatory cells infiltration between fragment muscle fiber, In the lung section revealed inflammatory cells infiltration in the interstitial tissue with calcium particles deposition in the atrial tissue in addition to hemorrhage in the parabronchial tree in another animal.



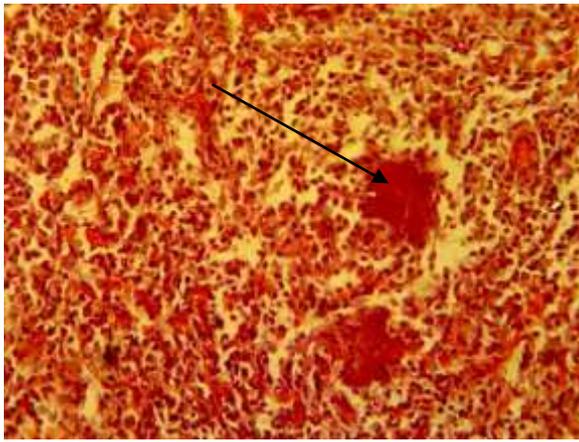
**Fig. 9.** Section in the heart of chicken in G3, fed contaminated diet at 24hr post infection with *S.typhimurium* shows fibrin deposition, heterophils infiltration, edema and hemorrhage in the pericardium in addition to inflammatory cells infiltration between cardiac muscle fibers (arrow) (H &E stain 400X)



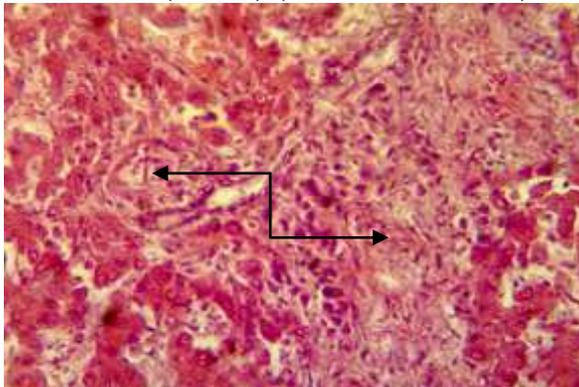
**Fig. 10.** Section in the kidney of chicken in G3 fed contaminated diet at 24hr post infection with *S.typhimurium* shows hemorrhage between renal tubules which suffering from a acute cellular degeneration (arrow) (H &E stain 400X)



**Fig. 11.** Section in the bursa Fabricius of chicken in G3, fed contaminated diet at 3 days post infection with *S.typhimurium* shows severe depletion of lymphoid follicles with moderate hyperplasia of bursal epithelium and cystic formation in high magnification (arrow) (H &E stain 400X).

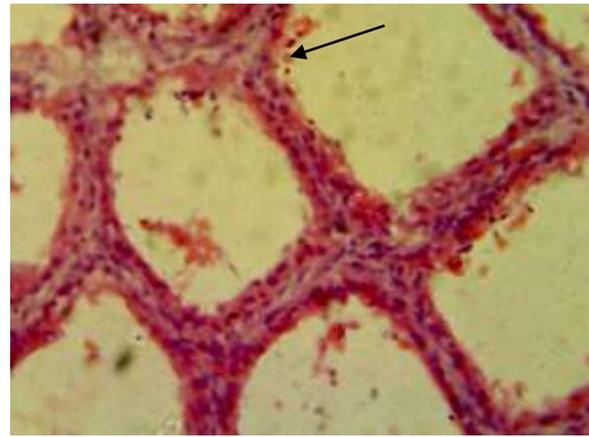


**Fig. 12.** Section in the spleen of chicken in G3, fed contaminated diet at 24hr post infection with *S.typhimurium* shows severe depletion of white pulp with bacterial colonies(arrow) (H &E stain 400X)

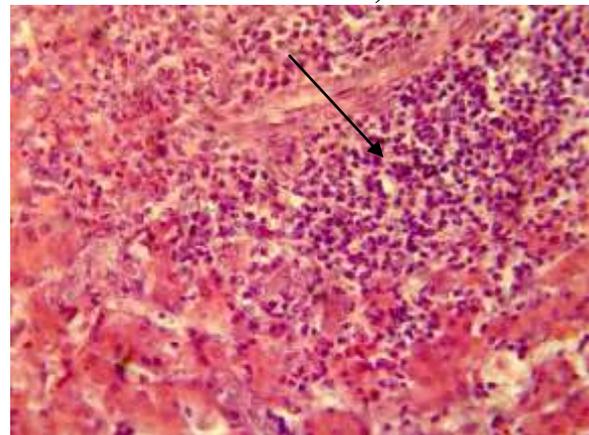


**Fig. 13.** Section in the liver of chicken in G3, fed contaminated diet at 1 week post infection with *S.typhimurium* shows severe portal fibrosis of liver parenchyma (arrow) (H &E stain 400X)

**G4 control - (without treatment)**  
Histopathological examination revealed that the spleen expressed normal white pulp with mononuclear cells lining sinus of red pulp, and the bursa showed folded mucosa, epithelial layer, cortex and medulla of lymphoid follicles, also it was recorded that the lung normal with parabronchial tree lining by single epithelial cells and atria around alveolar duct (Fig.:14), mononuclear cells aggregated in portal area of the liver (Fig.:15), few mononuclear cells were seen between mucosal glands of intestine, in addition to normal skeletal muscular fiber and normal structure of the kidney and heart.



**Fig.: 14.** Section in the lung of animal in G4, without any treatment at 3 weeks, shows single epithelial layer and LP of parabronchial branches (arrow) (H &E stain 400X).



**Fig.: 15.** Section in the liver of animal in G4, without any treatment at 3 weeks, shows mononuclear cells aggregation in portal area (arrow) (H &E stain 100X)

The present study showed increased thickness of the skin in chicks immunized with WSSAg, this result may indicate that this type of antigen can stimulate cell-mediated immune response, since DTH reaction was considered one sign of cell-mediated immune response that stimulated by Th1 cytokines such as IFN- $\gamma$ , this idea was in agreement with (20) who demonstrated that WSSAg stimulated both humoral and cell-mediated immune response (24). The current result revealed that immunized chicks with WSSAg and fed diet contaminated with mycotoxins expressed low skin thickness and serum antibody titers as compared with those values in the immunized chicks with WSSAg only, these results may indicate that the mycotoxin can cause suppression of humoral and cell-mediated

immune response which is dependent on activation of CD4 T cells by proper presenting antigen cells APCs, these idea was agreement with (19) who demonstrated that aflatoxin B1 (AFB1) can induce dysregulation of the antigen presenting capacity of dendritic cells associated with impair cell mediated immunity. The mean value of phagocytic index in G2 was higher than the other group this result may indicate (WSSAg) stimulate immune response through production interferon gamma that stimulate activity of macrophage this idea agreement with (12) who was reported both of INF- $\gamma$  and TNF- $\alpha$  can stimulate the bactericidal activity of macrophages. Also the phagocytic activity in G1 were less than G2 this result indicate that mycotoxin can suppress activity of phagocytic cell. This idea agreement with (5) who demonstrate the mycotoxin can decrease innate and acquired immune response. The high mortality rate in G3 post infection with *S.typhimurium* may indicate that the isolated strain highly virulent and avoid the immune system and disseminated to all the organs causes septicemia and dead of animal this idea agreement with (11) who demonstrated infection with salmonella particularly *S.Gallinarum*, progresses anaemia and septicaemia occur with salmonella shed back result in death of the animal. Also the highly mortality 80% of G1 and G3 may indicate that the mycotoxin increase susceptibility to infection to *S. Typhimurium* as result to depression of immune response and produce oxidative stress this idea in coincidence with result of immune response, results this idea was agreement with (30) who recorded that mycotoxins can suppress immune responses and result in increased susceptibility to infection, also high mortality rates in broiler chickens in group four as compared with immunized animals fed normal diet may indicated that the mycotoxin can inhibit immune response elicited by WSSAg, these idea was agreement with (14) and (14) who demonstrated that T-2 can inhibit protein and DNA synthesis and immunosuppression associated with impair antibodies production and inhibit DCs maturation and development else, it was recorded that T 2 can decrease function of the innate immune response (13).

The severe pathological lesions in the examined organs of chicks fed diet contaminated with mycotoxin may due to cytotoxic effect of the mycotoxins ,that cause liver and kidney damage (23) that associated with increase susceptibility of bird to infection (10) as well as cellular apoptosis (2), also these mycotoxin can cause oxidative damage of the cells consist such as lipid, proteins and nucleic acids (18), in addition to it was recorded that T-2 can reduce T-lymphocytes proliferation and antibody production (21). The severe lesions in the lymphatic tissue associated with dead most of broiler fed diet contaminated with mycotoxin after infection with *S.typhimurium* may indicated that this toxin act as predisposal factor for *Salmonella* infection , these idea was agreement with (17) who recorded that the poultry fed diet contaminated with T 2 expressed severe economic losses as a result infection by different disease outbreaks, It was recorded that the spleen was more sensitive to AFB1 as a result that this toxin cause severe damage to lymphoid tissue (6). The depletion of bursa of fabricia in the broiler infected with *S.typhimurium* in the present study may indicated that the toxin of this pathogen cause damage of these organs ,this idea was agreement with (25) who found that various infectious disease such as *E.coli* and *Salmonellosis* can induced acute atrophy these organs.

## REFERENCES

1. Abrahams G.L. and M. Hensel, 2006. Manipulating cellular transport and immune responses: dynamic interactions between intracellular *Salmonella enterica* and its host cells. *Cellular Microbiol.* 8(5) pp: 728–737
2. Afsah-Hejri L.; S. Jinap; P. Hajeb; S. Radu and SH. Shakibazadeh, 2013. A review on mycotoxins in food and feed Malaysia case study. *Compr Rev Food Sci F.* 12: 629–651
3. Al-Chalabi D. A.; A.A. Ali and F. M. Hussein, 2017. Diagnostic environmental monitoring system in a poultry house. *The Iraqi J. of Agr. Sci.* (3) : 860-873
4. Ali N.; K. Muñoz, and G.H. Degen, 2017. Ochratoxin A and its metabolites in urines of German adults—an assessment of variables in biomarker analysis. *Toxicol. Lett.* 275:19– 26

5. Aupanun S.; S. Poapolathep; M. Giorgi; K. Imsilp and A. Poapolathep, 2017. An overview of the toxicology and toxicokinetics of fusarenon-X, a type B trichothecene mycotoxin. *J. of Vet. Med. Sci.* 1: 6–13
6. Batra P.; A.K. Pruthi and J.R. Sadana, 1991. Effect of aflatoxin B1 on the efficacy of turkey herpes virus vaccine against Marek's disease. *Res. in Vet. Sci.* 51: 115–119.
7. Carroll K.C.; J. H. Jorgensen and M. A. Pfaller 2015. American Society for Microbiology. *Manual of Clinical Microbiology*. 11<sup>th</sup> ed. Washing, DC: ASM. pp: 41.-151
8. CDC, 2014b. *Salmonella*. Atlanta, GA. Available-online at: <http://www.cdc.gov/salmonella/> (accessed June 21, 2016).
9. Cegielska-Radziejewska R.; K. Stuper and T. Szablewski, 2013. Microflora and mycotoxin contamination in poultry feed mixtures from western Poland, *Ann. of Agri. and Env. Med.* 1: 30–35
10. Chang C.F. and P.B. Hamilton, 1991. Mycotoxicosis. In *Disease of Poultry*, 9th ed.; Calnek, B.W., Ed.; Wolfe Publishing Ltd.: London, UK, pp: 893–897
11. Chappell L.; P. Kaiser; P. Barrow; MA. Jones; C. Johnston and P. Wigley, 2009. The immunobiology of avian systemic salmonellosis. *Vet Immune I Immunopathol.* 128: 53–59.
12. Denis M., 1991. Modulation of mycobacterium lepraemurium growth in murine macrophages: beneficial effect of tumor necrosis factor alpha and granulocyte-macrophage colony-stimulating factor. *Inf. and Imm.* 2: 705-707
13. Guilford F.T. and J. Hope, 2014. Deficient glutathione in the pathophysiology of mycotoxin-related illness. *Toxi.* 6: 608-623
14. Hossam E.D.M.O., 2013. Mycotoxins-induced oxidative stress and disease, *Mycotoxin and Food Safety in Developing Countries*. Makun, H.A. InTech, Croatia. pp: 63–92
15. Kshash Q. H., 2008. The Role of some antigens of E-coli isolated from bovine mastitis in the protection of mammary gland in Mice. Ph.D. Dissertation, College of Vet. Medicine, Baghdad University
16. Majowicz S. E.; J. Musto; E. Scallan; F. J. Angulo; M. Kirk, and S.J. O'Brien, 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin. Infect. Dis.* 50: 882–889.
17. Malik G.; J. P. Dhahiya; G. Sandeep and S. K. Mishra, 2002. Clinicopathological studies on chlorpyrifos intoxication in broiler chicken. *Proceedings of 19<sup>th</sup> Annual Conference of Indian. Ass. Of Vet. Pathol.* pp: 26-28
18. Mary V.S.; M.G. Theumer; S.L. Arias and H.R. Rubinstein, 2012. Reactive oxygen species sources and biomolecular oxidative damage induced by aflatoxin B1 and fumonisins B1 in rat spleen mononuclear cells. *Toxicol.* 302: 299-307
19. Mehrzad J.; B. Devriendt; K. Baert and E. Cox, 2014. Aflatoxin B1 interferes with the antigen-presenting capacity of porcine dendritic cells. *Toxicol Vitro.* 28: 531–537.
20. Mohan A.M., 2019. Immunopathological study of *Klebsiella pneumoniae* as major ESKAPE Pathogens in Al Qadisiyah Province. Ph.D. Dissertation, College of Vet. Medicine, Baghdad University. pp: 55-57
21. Obremski K.; P. Podlasz; M. Żmigrodzka; A. Winnicka; M. Woźny; P. Brzuzan; E. Jakimiuk; P. Wojtacha; M. Gajęcka; L. Zielonka and M. Gajęcki, 2013. The effect of T-2 toxin on percentages of CD4+, CD8+, CD4+CD8+ and CD21+ lymphocytes, and mRNA expression levels of selected cytokines in porcine ileal Peyer's patches. *Pol J Vet Sci.* 16: 341-349
22. Old D.C. and E.J. Threlfall, 1998. *Salmonella In: Microbiology and Microbial Infection* "9<sup>th</sup> ed. Oxford University. New York
23. Pappas A.; E. Tsiplakou; M. Georgiadou; C. Anagnostopoulos; A. Markoglou; K. Liapis and G. Zervas, 2014. Bentonites binders in the presence of mycotoxins: Results of in vitro preliminary tests and an in vivo broiler trial. *Appl. Clay Sci.* 99: 48–53.
24. Pe' Rez C.; G. M. Caldero' N; C. Xime' Nez and E. I. Melendro, 1996. Human cell-mediated immune responses to antigenic fractions of *Salmonella typhi*. *Immunol.* 89: 262–267
25. Sadeghi A.A.; P. Shawrang and S. Shakorzadeh, 2015. Immune response of *Salmonella* challenged broiler chickens fed

diets containing gallipro, a bacillus subtilis probiotic. Prob. Antimicrobial Prote.7: 24–30.

26. Sangdee K.; P. Thummabenjapone and A. Sangdee, 2012. Evaluation of antigen preparation methods for polyclonal antibody production against streptomyces spp. Brit. Microbiol Res. J. 3:137

27. Shlej1 A. A.; J. M. Saaid and K. M. Thlij, 2015. Ability of lactobacillus actobacillus casei and lactobacillus acidophilus to protect against the toxicity in broilers fed contaminated diet aflatoxin B1. The Iraqi J. of Agr. Sci. 46(4): 652-661

28. Tayeb1 T.; N. H. R. Artoshi 1 and B. Sögüt, 2019. Performance of broiler chicken fed different levels thyme, adiantum, rosemary and their combination. Iraqi J. of Agr. Sci. 50(6):1522-1532

29. USDA-FSIS, 2014. Progress report on Salmonella and Campylobacter testing of raw meat and poultry products, CY 1998–2013

30. Valtchev1 I.; T. Koynarski2; L. Sotirov2; Y. Nikolov1 and P. Petkov, 2014. Effect of aflatoxin B1 on moulard duck's natural immunity. Pak. Vet. J., 1: 67-70.