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Evaluation of the effect of chitosan on the control of *salmonella typhimurium* infection in broiler chickens

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Abstract

Antimicrobial activities of chitosan against ***Salmonella typhimurium*** were assessed through conducting an experiment on eighty, one day old Hubbard broiler chicks were divided equally into four groups (twenty for each). First group was kept as control group fed *ad libtum* without any special fed treatment, second and third groups were fed on diets containing chitosan (**0.1% kg diet**) from the first day of age, while both third and fourth group were challenged orally with **2** ml(**1800x10^5**) ***S. typhimurium*** on sixth day of age or experimental start**.** Challenged fourth group had got no treatments; the experimental period lasts fifteen days where specimen collection began. ***Salmonella typhimurium*** used in infection induction was isolated from naturally diseased and freshly dead field cases then identified traditionally by morphological and biochemical methods with running the sensitivity test on the isolated strain against some antibiotic of choice and chitosan. Re-isolation of the challenged bacteria was done at the end of experiment to confirm the positivity of induced infection. Specimen from blood for hematological, and serum for testing nitric oxide, cholesterol, IgM, IgG and triglyceride levels were done with recorded clinical signs and applied postmortem examination then collected tissues samples from livers and intestines for histopathological examination. The obtained results showed ameliorative effects on both blood and tissue in the treated group with chitosan when compared with infected non-treated group indicating the antibacterial feature of chitosan that improve the immunological status of the broiler chicks.

Keywords*: Salmonella typhimurium, chitosan, broiler chickens.*

Introduction

Antibiotic-resistant bacteria is considered major problem world wide especially when antibiotics are used for the production of food animals. Therefore, immune stimulants may be extremely useful for increasing the immune competence **(Guo *et al.,* 2003)**.

The antimicrobial activity of chitosan against different groups of microorganisms, such as bacteria, yeast, and fungi, has received considerable attention in recent years **(Limam *et al.,* 2011).** Chitosan is characterized by high antibacterial and fungicidal activities and protected the chickens against *Salmonella* infection. **(Ballicka *et al.,* 2005)**.Moreover reduced the incidence of diarrhoea and alleviated some other signs associated with infection (**Liu *et al.,* 2010**).

Chitosan, deacetylated chitin, is a natural alkalinepolysaccharide recently recognized as one of prebiotics, and it is widespread in nature. The exoskeletons of crustacean family are good sources of chitosan. Chitosan enhance cellular and humoral immune response, and stimulating macrophages to produce interleukin-1 **(Zaharoff *et al.,* 2007).**
Chitosan effect on lipid metabolism by decreasing blood levels of triglycerides and total cholesterol and improving performance and meat quality **(Tang *et al*., 2005)**. Nitric oxide

(NO) consider an important factor in immunity, which play an important role in modulating immune responses and inflammation, and it was synthesized from L-arginine by inducible nitric oxidesynthase (iNOS) **(Korhonen *et al.*, 2005**). Chitosan has been shown to enhance the content of NO and expression of iNOS in rat macrophages and this associated with increased NO secretion and expression of iNOS **(Porporatto *et al.*,2003).**

Pathological alterations in freshly dead birds due to *S. typhimurium* infection grossly were liver discoloration and enlargement, thickening and inflammation of intestinal mucosae with necrotic foci on the intestinal surface while, microscopically liver showed congestion, haemorrhage, focal degeneration and necrosis, inflammatory cells infiltration locally at per vascular areas and thrombin central vein. Hepatocytes with hydropicvacuolation. Complete necrosis in some areas of the hepatic parenchyma. Dilatation of sinusoids, thickening of liver capsule while, intestine showed desquamation of mucosal epithelium where the lumen filled with necrotic masses. Severe infiltration of inflammatory cells with atrophied intestinal glands and necrosis **Muna *et al.*, (2016) and Chiroma *et al.*, (2017)**

Aim of the work

The goal of this study was to explore the protective effect of chitosan as a natural antibacterial alternative against S. *typhimurium* infection in chickens sense antibiotic resistance issue was recognized ,as well as testing the impact of chitosan on immunological status, blood features, and tissues architecture

Materials and Methods

I-Pre-experimental stage

Sampling and Isolates Characterization

A total of **52** intestinal samples were aseptically collected from diseased and freshly dead broilers suffering from diarrhea. All samples were subjected to conventional methods for isolation and identification according to **(Quinn *et al.*, 2002).** Identified isolates were serotyped by a standard slide and tube agglutination test in serology unit using commercial polyvalent and monovalent O and H antisera**. (Kauffman, 1974).**

Chemicals

Chitosan: (chitin deacetylation 93%) Oxford Lab Chem. Formula: $C_6H_{11}NO_4X_2$ (W.161.16) and purchased from sigma company. Chitosan was added on the diet in a dose of (**0.1%**) from the first day of age till the end of the experiment (**15** days).

Determination of antibacterial activity of chitosan

Agar well diffusion method was used as described by **Chung *et al.*, (2004).** Chitosan was accurately quantified and added to 0.25% acetic acid. Bacterial cultures were emulsified in normal saline and turbidity was matched with 0.5 McFarland turbidity standards. 0.1mL of RVs broth culture of the Salmonella strains were firmly seeded over the Mueller-Hinton Agar (MHA) plates. Wells of 6 mm diameter was punched over the agar plates using a sterile cork borer. Using a micropipette, solution of chitosan (**0.1%**) was added to different wells in the plates. These plates were incubated at 37 ºC for 24 hours. Absence or presence of inhibition zones, as well as their diameters, were recorded. All the results were compared with the standard antibiotic discs of Doxycycline (**30µg**), colistin (**10µg**), ciprofloxacin (**5) µg**), neomycin (**30 µg**), cefadroxil (**30 µg**), methicillin (**51 µg**), enrofloxacin (**5 µg**) gentamycin (**10 µg**) penicillin (**10 µg**) and chloramphenicol (**30 µg**).

II-Experimental design

This study was conducted on eighty, one day old Hubbard broiler chicks, feed and water were supplied ad-libitum. Chicks were fed on a basal diet without or with some treatments and were divided into four equal groups (**20** birds each) and treated as shown in **table (1)**

Post experimental re-isolation

After slaughtering, caecal tonsils from four birds were sampled and pooled by intestinal segment were enriched in 10mL of RVs broth overnight at 37°C, *Salmonella typhimurium* were re-isolated in appropriate specific culture medium (XLD agar)

Blood sample

Blood samples were collected from wing vein puncture under aseptic precautions from fifteen

chicks in each group at 15 days. The first sample was 1 ml of blood collected on EDTA for hematological examination. The second blood sample was 3 ml of blood taken without anticoagulant in a clean and dry centrifuge tube, left to clot at room temperature and centrifuged at 3000 rpm for 5 min for serum collection. Serum samples were putted in dry clean capped tubes and kept in deep freeze at-20ºC for biochemical analysis.

The hematological and biochemical study

Erythrocytes (RBC), White blood cells count (WBCs) and white blood cell differential were calculated using the method according to **Feldman *et al*., (2000)**. Hemoglobin (Hb) concentration was estimated according to **Blaxhalland Daisley (1973)**. Serum samples were used for determination of Immunoglobulins M (IgM) according to **Naito (1986)**, While immunoglobulins G (IgG) were measured according to **Murray *et al*., (2009)**. Serum nitric oxide (NO) was analyzed using nitric oxide assay kit (ab65328) purchased from Sigma company. Cholesterol was performed according to **Tietz, (1995)**. Triglyceride was determined according to **Buccolo, (1973)**.

Pathological examination

Tissue specimens*:*

Specimen from liver and intestine were collected from sacrificed chicks at the end of experiments (at **15th** day of age) and fixed in **10%** buffered neutral formalin. Paraffin sections **5** micron thick were prepared and stained with hematoxylin and eosin stain **(*al*., 2012)** and examined microscopically. Recording the lesions score of the previously collected chickens from third and fourth groupsat the end of the experiment. Scoring the histopathological changes were evaluated as mild (+), moderate (++) and severe (+++)

Statistical analysis:

Statistical analysis was performed using the analysis of variance (ANOVA). Duncan's Multiple Range **Duncan, (1955)** was used to determine differences among treatments mean at significance level of 0.05. All statistics were run on the computer using the SPSS program **(SPSS, 2004).**

Results and Discussion

I- Bacteriological results

The most prevalent species was *Salmonella T yphimurium*, where 12 isolates had been identified. Serological typing was done by using both poly and monovalent antisera, which revealed that 8 isolated *Salmonella* was belong to serovar *S. Typhimurium*,3 isolates *S. Kentukey*, and one isolates *S. Enteritidis*. *S. Typhimurium* continues to be among the most common serovars isolated from poultry and a common cause of human *salmonellosis*. **(Foley *et al.,* 2011).** Chickens pretreated orally with chitosan were resistant to *Salmonella* infection,

as a result of the effect of chitosan on the mechanisms acting at the early phase of defense system such as accumulation and activation of macrophages, activation of natural killer cells, and induction of interferon **(Nishimura *et al.,* 1986)**. Also due to antibacterial activities against *salmonella.* **Balicka *et al.,* (2005)**. The clinical symptoms and histopathological changes in chickens infected with bacteria and treated with chitosan (**group3**) were mild in comparison with those infected with *Salmonella* and not treated (group4). Our results agree with, **Iida *et al.,* (1987**) who reported that the protection of the host against bacterial infection is stimulated by chitosan. Also, **Ballicka *et al.,* (2007)** concluded that orally administered chitosan protected the chickens against *Salmonella gallinarum* infection. Moreover, It has been documented that chitosan it self has antimicrobial activity and exerts antimicrobial activity against bacteria and fungi **(Dongwei *et al.,* 2009**). In group. **(4), 80%** of birds had clear clinical symptoms, especially diarrhea and depression, loss of appetite with moist congested vent , all were correspond well with the results obtained by **Herich *et al.,* (2004).** Moreover, **Huang *et al.,* (2007)** suggested that the prebiotic effect of chitosan could be related to a chitosan attachment to the bacteria, leading to an immune response to this antigen, or by direct stimulation of the immune system. Therefore, immune response enhancement by chitosan may be of a practical importance in improving immunization programmers in poultry production. **Ballicka *et al.,* (2008).** In addition**, Nelson *et al.,* (1994)** observed a decreased number of bacteria in the caecum, mesenteric lymph nodes, and livers of

mice fed dietary chitosan also. In the present investigation the antibacterial activity was articulated as inhibition zone diameters (IZD) measured in mm of samples against the *Salmonella* strains based on the well diffusion assay as table (2). Chitosan showed maximum inhibition zone with *Salmonella serotypes.* diameter of **22-25** mm at concentration of 0.1 ml /well, but recorded the lowest inhibition zone (**7mm**) inhibition zone was recorded against *Salmonella spp*. in phosphorylated chitosan **(Shanmugam *et al.,* 2016).** Chitosan could be a good source of drugs that may be used against bacterial infection. Moreover, diffused antibacterial activity of chitosan, motivated the clearing zone of the bacterial growth (**Osiris *et al.*, 2016).**

Chitosan extracted from prawn shell showed antibacterial activity against *Salmonella typhi***. (Reni and Sivakumar, 2017).** In comparison with ourdata, chitosan show efficient antimicrobial assay especially against S. *serotypes* with maximum inhibition zone was (**25**mm) (**Hameed *et al.,* 2019).** Similarly, chitosan showed inhibition zone with *Salmonella* serotypes diameter of 18- 23mm at different concentration, **Warda *et al.,* (2019).** According to **Monarul *et al.,* (2011)**. The highest zone of inhibition against *Salmonella Paratyphi* were found **12-16** mm and chitosan have excellent antibacterial activity against *Salmonella spp*. But the lowest activity with**7**mm inhibition zone was recorded against *Salmonella spp*. in phosphorylated chitosan **(Shanmugam *et al.,* 2016).**

In contrast to **Benhabiles *et al.,* (2012**) reported that Chitosan exhibited a bacteriostatic effect on all bacteria tested, except *Salmonella typhimurium*and *S. typhi.* were the most resistant bacteria strain studied. chitosan showed maximum antibacterial activity against *Salmonella* **Figure(1)** maximum inhibition zone diameter recorded 24mm at concentration 0.2% and the lowest inhibition zone6 mm at concentration **0.025%** and negative zone at concentrations **0.0125%** and **0.00625%.** The present results fall in line with the findings of **Shanmugam *et al.,* (2016)** recorded that chitosan was exhibited concentration dependent antibacterial activity and the zone of inhibition varied with the concentration. **Monarul *et al.,* (2011)** indicate that there exist a linear relationship between dose used and zone diameter **table (3).**

II-Hematological and Biochemical results

Hematological changes in the Salmonella infected chickens in this study presented in **table (4),** the results revealed a significant decrease in packed cell volume (PCV), haemoglobin concentration and RBCs count in infected non treated chicken, our results in accordance with that of **Prasanna and Paliwal, (2002) and Chiroma *et al*., (2017)**, this decline may be due to salmonella endotoxin which suppress haemopoietic activity **(Assoku and Penhale, 1970)** or depletion in vitamin B12 due to hepatic dysfunction **(Feldman *et al.,* 2000).** In the present study an increase in total leukocyte counts in infected birds comparing with control, this result in accordance with **Kokosharov, (2002)**, the increase in leukocyte may be due to acute and chronic inflammatory lesions and tissue necrosis caused by salmonella endotoxin. Treatment of infected birds with chitosan resulted in a significant increase in hematological parameters. Our results approved with **Yan *et al.,* (2010)** reported that dietary chitosan improved the concentration of RBC and WBC. Also, **Meng *et al.*, (2010) and Meshkini *et al*., (2012)** reported that animals treated with chitosan evoked increase in the number of white blood cells and lymphocyte percent. This increase in the number of WBC due to the fact that chitosan binds to bacteria and initiate the host immune system response, subsequently increase WBCs, another point of view is related to chitosan has active amino and hydroxyl groups, these groups activate macrophages to induce antibodies and subsequent increase WBC **(Nuengjamong and Angkanaporn, 2017)**. Concerning the effect of salmonella infection on serum humoral responses, infected chicks with *salmonella* evoked significant increase in IgM and IgG comparing with control, this result in accordance with **Salisbury *et al.,* (2014).** Chicks treated with chitosan evoked a significant increase in IgM and IgG, this result approved with **Huang *et al*.,(2007)** observed an increase in serum level of immunoglobulin in chicks supplemented with chitosan. **Huang *et al*., (2005)** suggesting that the increase in immuno-

globulin may be related to cytokine production and reported that dietary chitosan increases immunoglobulin synthesis by B lymphocytes by increase the availability of circulating amino acids. Moreover **Zaharoff *et al.,* (2007)** mentioned that chitosan has immunostimulatory properties because it contains reactive and functional groups (amino acids and hydroxyl groups) which stimulate humoral immune response. Nitric oxide (NO) is recognized as an important factor in nonspecific immunity, NO is also a great inflammatory mediator and it considered as a cytotoxic agent and modulates immune responses and inflammation **(Korhonen *et al*., 2005)**. In the present study, **table (5)** the NO level in salmonella infected birds was suppressed, our results in accordance with **He *et al.,* (2012),** the suppression in NO may be due to effect of protein secretes by salmonella **(Das *et al.*, 2009)**, moreover, salmonella produce enzymes, including flavor hemoglobin, flavorubredoxin, and cytochrome c nitrite reeducates which can suppress NO **(Mills *et al*., 2008).** In the present study, treatment of infected chicks with chitosan increase NO concentrations in serum, our results approved with **Peluso *et al.,* (1994) and Yu *et al.*, (2004),** moreover **Deng *et al.*, (2008)** mentioned that dietary supplementation of chitosan improve the immunity of broilers and improved serum NO content. This increase in serum NO in chitosan groups is due to increase the activity of inducible nitricoxide synthase (iNOS) in serum **(Li *et al.,* 2009).** The effect of chitosan on serum cholesterol and triglyceride as shown in **table (5)** chitosan decreased plasma cholesterol and triglyceride concentrations, our results in accordance with **Razdan *et al.,* (1997)** reported that chitosan bind to bile acids and, consequently reduce the concentration of duodenal bile acids, more over **Li *et al.*, (2007)** mentioned that chitosan decreased serumtriglyceride and total cholesterol concentrations through the reduction of lipid absorption in the intestine by binding bile acids, lead to increase cholesterol elimination rate.

III-Clinical signs, morbidity and mortality

General and enteric signs were appeared clearly only among chicks of the fourth group with 80% morbidity, depression with loss of appetite and variable degrees of marked mucoid yellow diarrhea resulted in dehydration and emaciation in some cases with congested vent, while mortalities was **15%,** these clinical signs, morbidity and mortality rate were so far parallel to those obtained by **Chiroma *et al*., (2017)** and corresponding well with **Herich *et al.,* (2004)** who noticed depression and huddling, ruffled feathers, somnolence, greenish-yellow diarrhea, loss of weight, a decrease in feed and water consumption, and sudden death with **100%** morbidity and **50%** mortalities. Little differences with that reference could be explained on a base of differences in dose of challenged strain and experimental period. Mild signs restricted in mild watery diarrhea, decreased body weight when compared to control were observed among chicks of the third group with **65%** morbidity and **5%** mortalities that could be attributed to the capacity of chitosan in diminishing the negative effect of *S. typhimurium* on infected chicks **(Ravi *et al.,* 2018),** while both the first and second Groups exhibited no visible signs or mortalities.

IV- Postmortem examination

At the end of the experimental period, the gross examination of the sacrificed chickens of the fourth group showed congestion of mesenteric and intestinal blood vessels with swelling of the intestine **(Fig. 2a)** which could be attributed to the liberated gases from salmonella strain, mucoid yellowish intestinal content was also observed**.** Incomplete yolk sac absorption **(Fig. 2b),** which was characteristic to some bacteria which *salmonella spp.* is one of them, pale enlarged liver with focal discoloration **(Fig. 2c),** these findings were in partial accordance with **(Muna *et al.,* 2016)** who observed some typical gross lesions and differ in other such discoloration and enlargement of liver, inflammation and thickening of intestinal mucosae. Necrotic foci on the hepatic surface. Severity of infection due to virulence and dose of strain with short experimental time could be the reason of that difference time**.** Milder macroscopic lesions were observed among chickens of the third group because of the ameliorative effect of applied chitosan on the negative effects of *S. typhimurium* on these infected treated chickens **(Arch and Dummerstorf, 2007),** while no detected gross lesions appeared on sacrificed chickens of both first and

second group

V-Histopathological results:
Chickens of the fourth group infected with *S. typhimurium* without treatments at the end of experiment showed marked and clear lesions in comparison with chitosan treated group demonstrated in both livers and intestines. Livers tissues exhibited some changes asperiductalcoagulative necrosis with cholestasis and mild vacuolation of ductal epithelium **(Fig. 3a),** some cases showed congested liver but most cases didn't**.** Focal to multifocal areas of leucocytic cells infiltrations was the common pathological lesion seen in case of bacterial infection (**Fig. 3b)**. Some cases showed periductal fibrosis with cholest as is with or without hyperplasia of ductal epithelium (**Fig. 3c)** peivascularleucocytic cells infiltrations with von kuppefer cells proliferation also observed (**Fig. 3d)** while intestines of chickens of the same group exhibited some pathological alterations as degeneration of the intestinal gland with or without interglandular edema (**Fig. 4a),** interstitial leucocytic cells infiltrate both the intestinal mucosa and submucosa was seen that explained on a base of immunological response against challenged salmonella, extravagated blood accumulates in the submucosa and mucosa specially at the villus core (**Fig. 3b),** interglandularleucocytic cells infiltrate different parts of intestine such as the intestinal mucosa (**Fig. 4c)**, sloughing of intestinal mucosa with and some cases showed variable degrees of mucinous degeneration**.** Previously mentioned liver and intestine lesions were in harmoney with those obtained by some references **{Herich *et al.,* (2004). Muna *et al*., (2016)}. Chickens of group (3)** infected with *S. typhimurium* and chitosan treated exhibited mild to moderate lesions restricted in barely mild hepatic congestion and few focal areas of leucocytic cells infiltrations **(Fig. 5a)** while intestine of the same group revealed over wrinkles and meanders of the whole villus tips (**Fig. 5b)**, this mild lesions due to the effect of chitosan as antibacterial agent on chickens challenged with *S. typhimurium* which was explained well by **(Arch and Dummerstorf, 2007).** Detailed lesions and lesion severity in both third and fourth groups were mentioned in **table (5). Chickens of group. (2)** treated only with chitosan at the end of experiments exhibitednearly normal hepatic structure with mild sinusoidal dilatation (**Fig. 5c)** while intestine showing healthy normal structures with clear villus tips and non- adherent villi (**Fig. 5d).** Lesion**s** severity between two infected groups were showed in **table (6).**

Conclusion
Chitosan as a prebiotic substance has antibacterial activities revealed in its resistance to *salmonella typhimurium*in concentrations (0.2% - 0.025%)**,** this fact was confirmed by different studies including hematological, biochemical and pathological one ,all proved its role in stimulation of immune system of chicks and ameliorate the negative effect of bacteria (*S. typhimurium*) which detected clinically and pathologically.

Table (1). Experimental design

Treatments / Groups	*Salmonella Typhimurium* **2**ml **1800x10^6 cfu**	**Chitosan 0.1%/kg diet**
Group (1)	---	---
Group (2)	---	+
Group (3)	+	+
Group (4)	+	---

The chicks were infected orally with a suspension containing in **2**ml **1800x10^6** bacteria (on the McFarland scale) at **6th** day of age

Table (2). Antimicrobial activity of chitosan using agar well diffusion assay.

Treatments	**Con**	**Inhibition zone diameter (mm) against indicator Salmonella strains**											
		1	2	3	4	5	6	7	8	9	10	11	**12**
Chitosan	0.1%	15	19	10	20	12	11	9	9	7	25	7	**22**
Ciprofloxacin	5µg	14	20	23	17	20	24	22	23	15	27	14	**28**
Colistin	10 µg	9	6	10	9	11	7	12	-ve	8	11	-ve	**-ve**
Neomycin	30 µg	-ve	-ve	6	7	9	10	-ve	6	-ve	8	-ve	**10**
Doxcycline	30 µg	7	-ve	8	-ve	7	7	-ve	11	9	12	-ve	**8**
Cefadroxil	30 µg	12	8	12	16	-ve	8	10	-ve	-ve	14	20	**14**
Methicillin	5mg	-ve	6	-ve	12	8	-ve	8	8	-ve	14	7	**-ve**
Enrofloxacin	5 µg	11	18	12	16	22	20	19	11	12	25	16	**27**
Gentamycin	10 µg	12	18	13	8	14	16	12	9	10	16	11	**18**
Pencillin	10IU	11	26	12	-ve	18	20	14	-ve	-ve	12	16	**23**
Chloramphenicol	30µg	-ve	-ve	6	8	-ve	6	17	8	11	9	13	**18**

Table (3). Antimicrobial activity and zone of inhibition of chitosan (mm) against Salmonella

Concentration of chitozon	**0.2%**	**0.1%**	**0.05%**	**0.025%**	**0.0125%**	**0.00625%**
Zone of inhibition in diameter (mm)	24	22	9	6	-ve	**-ve**

Table (4). The effect of chitosan on erythrogram and leukogram of clinically healthy and infected chickens with salmonella

Groups	**RBCs (10^6×mm3)**	**Hb (g/dL)**	**PCV%**	**WBCs (10^3×mm3)**	**Lymphocyte%**	**Hetrophil%**
Group 1	2.8±0.018a	8.7±0.12a	26±0.2a	26.5±0.23d	31.6±0.88b	64±0.57c
Group 2	2.7±0.027a	8.5±0.12a	25.8±0.17a	30.3±0.88c	36±1a	62.6±0.88c
Group 3	2.3±0.12b	6.9±0.11b	23±0.57b	34.3±0.88b	28.3±0.88 c	68±0.57b
Group 4	1.4±0.11c	5.1±0.08c	17.6±0.33c	46.3±0.33a	21.6±0.85d	76.6±0.88a

RBCs: Red blood corpuscle Hb: Hemoglobin PCV%: Packed cell volume WBCs: White blood corpuscle. Means with different letters at the same column were significant P<0.05

Table (5). The effect of chitosan on biochemical parameters of clinically healthy and infected chickens with salmonella

Groups	**IgM (gm/L)**	**Ig G (gm/L)**	**Nitric oxide (µmol/L)**	**Triglyceride, mg/dL**	**Total cholester-ol, mg/dL**
Group 1	3.6±0.15c	3.2±0.1c	11.7±0.14a	77.6±1.4a	124.3±1.4ab
Group 2	4.1±0.088b	4±0.11b	11.9±0.14a	70± 1.1b	121.3±1.8b
Group 3	4.3±0.18b	4.4±0.11a	11.1±0.17b	74.3±1.3a	124.6±1.4ab
group4	5.2±0.17a	4.8±0.088a	8.1±0.14c	80.3±1.6a	128±1.5a

Means with different letters at the same column were significant P<0.05

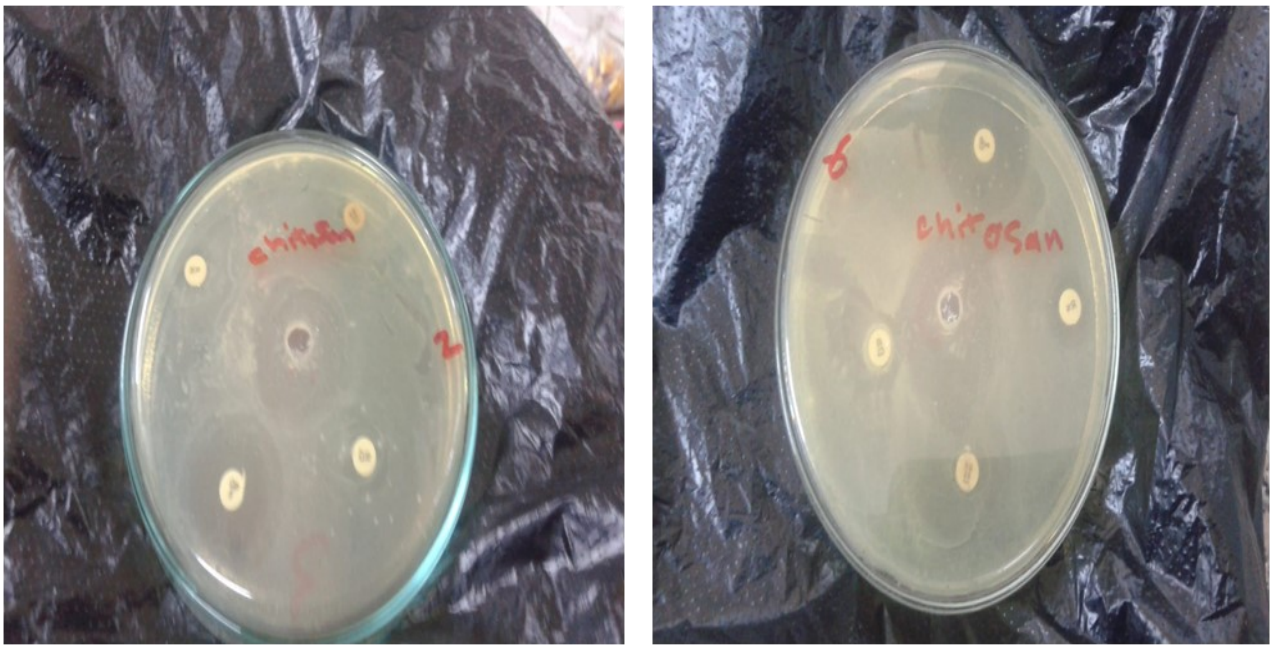

Figure (1). Inhibition zone produced by chitosan against two different isolates by using agar well diffusion

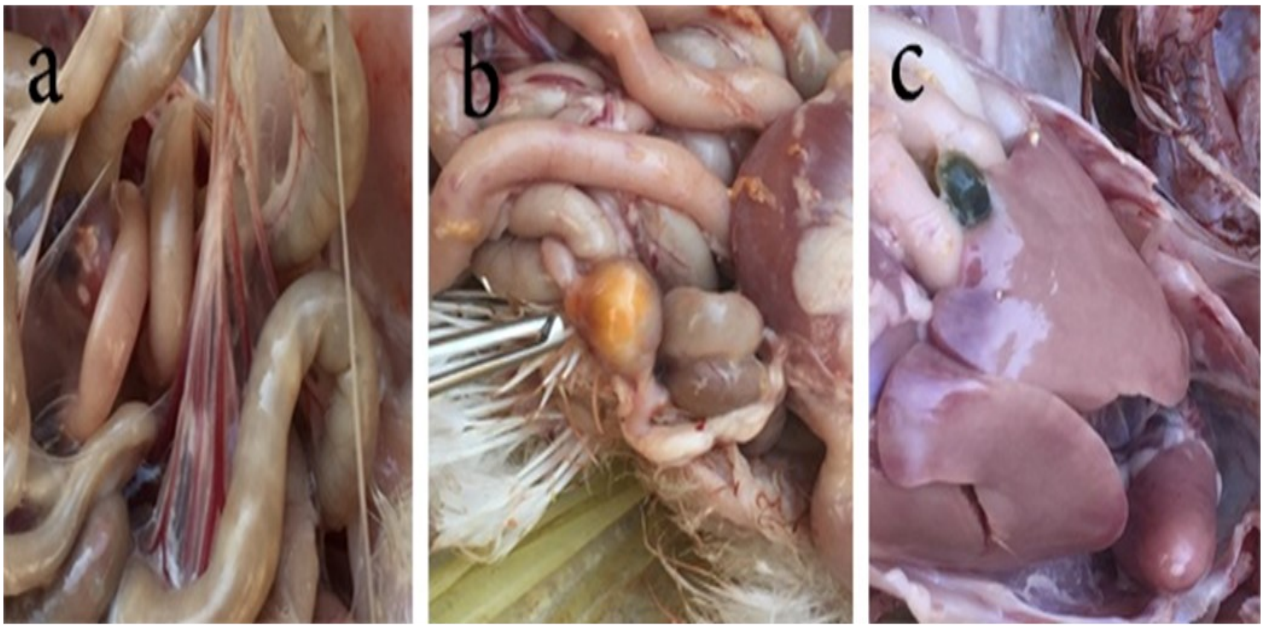

Figure (2): Chickens of group (4) infected with *salmonella typhimurium* and non-treated showing
(a): congestion of mesenteric and intestinal blood vessels with intestinal swelling
(b): incomplete yolk sac absorption
(c): pale enlarged liver with focal discoloration

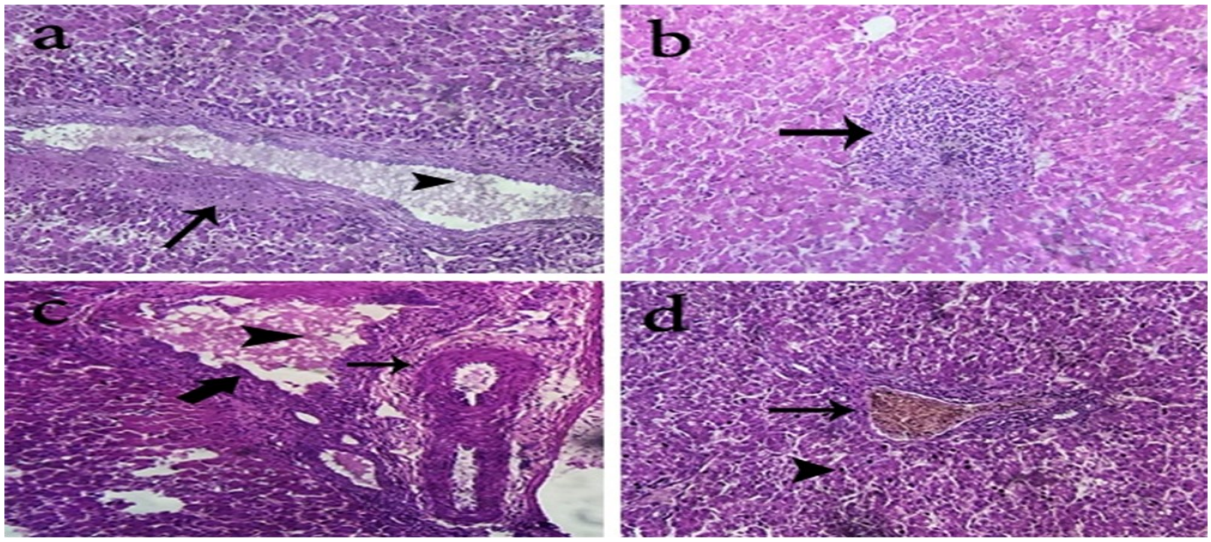

Figure. (3): Livers of chickens of group.(4) infected with *S. typhimurium* without treatment at the end of experiments showing.
(a): photomicrograph of liver showing periductalcoagulative necrosis with cholestasis and mild vacuolation of ductal epithelium **(H&E X100)**
(b): photomicrograph of liver showing focal area of leucocytic cells infiltrations **(H&E X 100)**
(c): photomicrograph of liver showing periductal fibrosis with cholestasis and hyperplasia of ductal Epithelium **(H&E X200)**
(d): photomicrograph of liver showing perivascularleucocytic cells infiltrations with von kuppefer cells proliferation. **(H&E X100)**

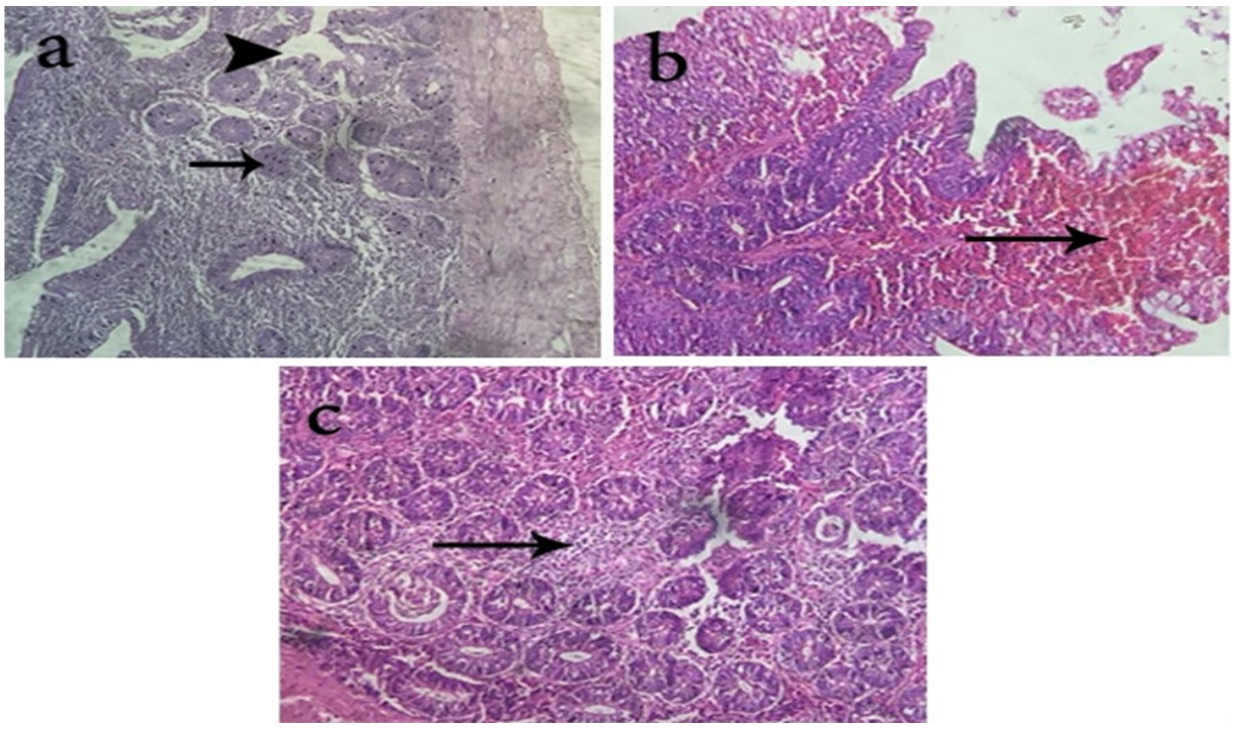

Figure (4): Intestines of chickens of group (4) infected with *S. typhimurium* without treatment at the end of experiment showing.
(a): Photomicrograph of intestine showing degenerated intestinal gland with interglandular edema **(H&E X200)**
(b): Photomicrograph of intestine showing extravagated blood accumulates in the villus core **(H&E X100)**
(c): Photomicrograph of intestine showing interglandular leucocytic cells infiltrate the intestinal mucosa **(H&E X400)**

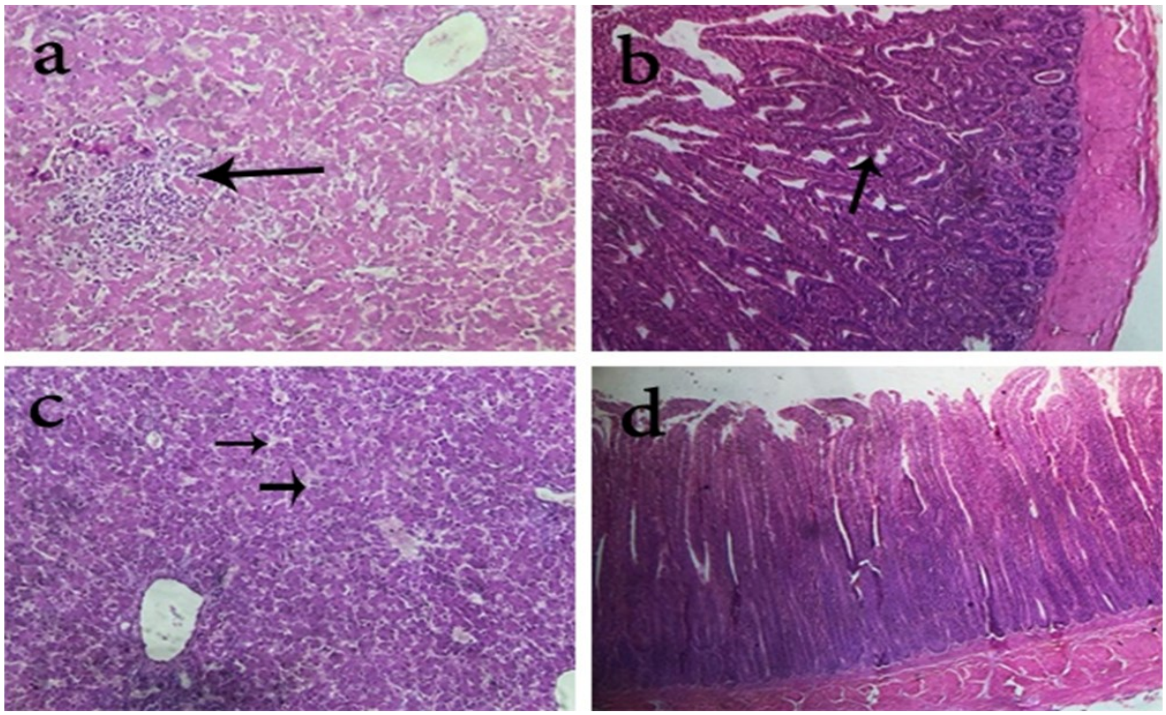

Figure (5): Chickens of group.(3)*infected with *S. typhimurium* and treated with chitosan and chickens of group (2)treated with chitosan at the end of experiments showing:**
(a)*: photomicrograph of liver showing focal interstitial leucocytic cells infiltration. **(H&E X200)**
(b)*: photomicrograph of intestine showing many meanders of the whole villus surface with mild atrophied intestinal glands .**(H&E X 100)**
(c):** photomicrograph of liver showing mild dilatation of sinusoidal capillaries **(H&E X 40)**
(d):** photomicrograph of intestine showing healthy normal structures with clear villus tips and infused villi**. (H&E X100)**

Table(6): Lesion scoreof the sacrificed chickens of the third and the fourth group

Groups / Lesions	Lesions score	
	Third group	Fourth group
Liver lesions		
unabsorbed yolk sac	+	++
pale enlarged liver	+	+++
focal hepatic discoloration	---	++
congested liver	+	++
coagulative necrosis	+	++
leucocytic cells infiltration	++	+++
fibrosis	+	+++
ductal epithelium hyperplasia	----	++
Intestinal lesions		
congested , swollen intestine	++	+++
yellow mucoid contents	---	++
yellow watery contents	++	+
gas bubble presence	+	++
degenerated intestinal glands	+	++
interglandular edema	+	++
haemorrhage	+	++
leucocytic cells infiltration	++	+++

mild (+), moderate (++) and severe (+++)

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