



## Study the Effectiveness of Chitosan on Experimental Infection of Chicks with Salmonellosis

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### Abstract:

This research attributed with chitosan as prebiotic and immunomodulator as well as to determine its effect on the body weights of experimental of chicks. The results had been showed increasing in body weights of chicks had been given chitosan.

The bacterial isolated revealed that chicks groups exposed to infection with *Salmonella typhimurium* and treated with chitosan before and with the same time of infection, these groups appeared a decrease in the bacterial isolation from intestines and liver in comparing with those exposed to infection without given chitosan

The passive hemagglutination test also revealed higher antibodies titers against *Salmonella typhimurium* in the same groups above in comparing with positive and negative control groups, so all these results detected the ability of this compound as inhibitor for bacterial growth and replication and to minimize their numbers as well as its effect and immunomodulator.

### دراسة استخدام مادة Chitosan على الإصابة التجريبية بـ *Salmonella typhimurium* في أفراخ الدجاج

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### الخلاصة:

هذا البحث تناول استخدام مركب chitosan باعتباره مركب prebiotic و immunomodulator محفز مناعي (محور مناعي) ضد جرثومة *Salmonella typhimurium* إضافة إلى محاولة معرفة تأثير الجرعة المستخدمة على أوزان الطيور (أفراخ التجربة).

حيث أظهرت النتائج زيادة ملحوظة في أوزان الأفراخ التي جرى معاملةتها بـ Chitosan كما وأظهرت نتائج الفحص الجرثومي أن مجاميع الأفراخ التي عرضت للإصابة بجراثيم *Salmonella typhimurium* وكانت سبق معاملةتها بـ chitosan أو عوملت أثناء إعطائها الجراثيم أظهرت انخفاضاً ملحوظاً في العزل الجرثومي من الأمعاء والأكباد مقارنة بالأفراخ التي أعطيت الجراثيم فقط بدون استخدام chitosan، كما أظهر فحص التلازن الدموي غير المباشر ارتفاعاً ملحوظاً في معايير الأجسام المضادة ضد *Salmonella typhimurium* في نفس المجاميع أعلاه مقارنة بمجاميع السيطرة الموجبة والسالبة.

وجميع النتائج المذكورة أعلاه أشارت إلى قابلية هذا المركب باعتباره مثبط لنمو الجراثيم وتقليل إعدادها إضافة إلى تأثيره باعتباره محفز مناعي.

**Introduction:**

Chitosan(B-C1-4) linked 2-amino-2 deoxy-D-glucose, is second naturally occurring biopolymer after cellulose which is the main structure component of crustaceans (crab, shrimp). Insects, mollusc and cell wall of certain fungus, and deacetylated from chitin (1). It is natural, positively charged polysaccharide and has a potential application in several areas including foods, pharmaceutical, biotechnology and environment, it is insoluble in water, but become soluble in acidic solution (2). Chitosan exhibits various biological activities including antimicrobial, antitumor, hemostatic action and acceleration of wound healing (3). Chitosan and its derivatives applied as a natural disinfectant against water borne pathogens (G+, G-) such as *Salmonella typhimurium* (4). The later was regarded as a main source of food poisoning human as well as its infections in poultry and other animals (5). Chitosan alters the intestinal microflora balance, inhibits the growth harmful bacteria, promotes good digestion and best immune function (6).

**Materials and Methods:**

*Salmonella typhimurium* strain: it was obtained from zoonotic disease unit. Inoculation into XLD and MacConkey agars and other biochemical tests has been done such as indole, H<sub>2</sub>S, Urease, phenylalanine and Simmons citrate. *Salmonella typhimurium* inoculated on brain heart infusion broth (18 hrs.) then cultured on brain heart infusion agar (heavy culture) for (24 hrs.) then using phosphate buffered saline PBS (pH: 7.2) the bacteria was harvested and then diluted according to MacConkey tube (No.3).

**Chitosan:**

Pure chitosan was selected from commercial product (Fat-Sorb) 500 mg/Capsule. This product was prepared by diluting one capsule contain 500 mg of active ingredient in a solution of citric acid in which each 1 ml contain 50 mg.

Different levels of chitosan (50, 25, 12.5, 6.25, 3.125 and 1.562) mg/ml were tested invitro and vivo (on bacteria cultured on the agar and by given it to the chicks with *S. typhimurium*) respectively.

**Experimental Design:**

The birds divided into five groups each one contain from ten birds.

- First group: Given *S.Typhimurium* culture alone, diluted according to MacConkey tube (No.3) (positive control group).
- Second group: Given 1ml of diluted chitosan then after four days given the bacteria prepared according to MacConkey tube (No.3).
- Third group: Given 1ml of diluted chitosan but the *S.Typhimurium* drenching had been done after 7 days.
- Fourth group: Given the diluted chitosan and the bacteria on the same time.
- Fifth group: Its present the negative control group, so it's neither chitosan given, nor bacteria drenching (its represent clean group).

**In vitro susceptibility of *S.Typhimurium* to the chitosan:**

This experiment was done by diluting the culture of *S.Typhimurium* according to MacConkey tube (No.3) and this distributed by sterile swab evenly into the brain heart infusion agar plate after cutting a wells in this agar of 5 cm diameter measuring. Then put the diluted chitosan in these wells as follows (50, 25, 12.5, 6.25, 3.125, 1.562) mg/ml. The dilution of both chitosan and bacteria had been done with PBS 7.2. Then the plate incubated in 37 °C for 24 hrs., the result had been read.

Two weeks later all birds have been sacrificed and culturing has been done from the liver and intestine (cecum), evaluate the salmonella occurrence in these organs in respect to chitosan drenching and to bleeding out to examine how could the chitosan may affect the situation in humeral immunity (antibody production) against *S.Typhimurium* by passive hemagglutination test. The birds also had

been weighted before killing them to weight.  
determine the effect of chitosan on the body

### Results:

**Table 1:** revealed the body weights of chicks for each group, which were recorded before killing the chicks.

Group	Body weight (gm)	Mean
First	413, 435, 392, 460, 430	426
Second	480, 420, 547, 600, 560	521.4
Third	545, 500, 520, 560, 535	531.8
Fourth	730, 506, 480, 603, 550	573.8
Fifth	350, 330, 420, 440, 435	395

**Table 2:** show the presence of bacteria after sacrificed birds and culturing from both liver and intestine.

Group	Occurrence of <i>S.Typhimurium</i> in liver	Occurrence of <i>S.Typhimurium</i> in cecum
First	+++	+++++
Second	-	++
Third	-	+
Fourth	-	++
Fifth	-	-

+ refer to less than 10 colonies appear.

++ refer to 10-20 colonies appear.

+++ refer to 20-30 colonies.

++++ refer to more than 30 colonies.

+++++ refer to heavy growth.

- refer to the no. appearance to any colony (clean culture).

**Table 3:** show the ability of the chitosan used in this experiment to inhibit the growth of the *S.Typhimurium* drenching to chicks but in vitro and by diluted the original dose given to birds.

Dilution of chitosan (mg/ml)	Dilution of the inhibition zone (cm)
50	2.75
25	2.35
12.5	1.90
6.25	1.50
3.125	1
1.562	No inhibition

**Table 4:** show the titer of antibody measured by passive heamagglutination test for each bird in all groups and the mean of the antibody titers for each group.

Group	Antibody titer	Mean
First	1/8, 1/8, 1/4, 1/4, 1/8	1/6.4
second	1/8, 1/16, 1/16, 1/16, 1/8	1/12.8
Third	1/8, 1/16, 1/16, 1/32, 1/32	1/20.8
Fourth	1/16, 1/16, 1/16, 1/32, 1/32	1/22.4
Fifth	1/2, 1/2, 1/2, 1/4, 1/4	1/2.8

The passive heamagglutination test had been done according to Stavitsky (7) and Al-Haddad (8).

### Discussion:

Table one concerning with body weights of birds referred that, there was no significant difference between first group and fifth one which represent, salmonella infection alone and the negative control respectively, but there was obvious difference between these two groups with second, third and fourth which given the bacteria plus chitosan, so there was no significant difference in weight between second and third groups which were given suspension of bacteria after four and seven days from chitosan drenching respectively but the fourth group differ from these two in which the chitosan plus bacterial suspension given of same time. So highest body gain occur in fourth group (573.8 gm) then the third one with (531.8 gm) and in the third class the second group with (521.4 gm), then the first one with (426 gm) and finally the fifth group with (395 gm).

So, in this research the dose of chitosan had been given to birds participated in weight gain in the three groups (2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup>) and this is may be due to the theory in which the influence of chitosan on weight gain depends on the dose and the route of administration (9), so we can explain the increasing in the weight of the groups treated with chitosan to the dose that was given and to route of administration which may represent low one (50 mg/ml) and the effect of given it orally directly with water but not with diet especially when we know that this

compound are fat absorbance and when we do the postmortem, the significant notice was the fatty changes in livers and hepatomegaly found in all these groups, and this agree with (10) who established that when chicken diets containing high ingredient from chitosan 30 g/kg from diet cause reduction in body weight and feed intake also (11) found that low viscosity chitosan supplement can decrease deposition of dietary fat but without reducing in food intake or body weight gain in broiler chickens in addition to that (9) detected that addition of 3% chitosan feed additive (high percent) had slightly decreased weight gain.

In table (2), we found that, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups show no any presence of *S.Typhimurium* in the liver while cecae appear from 10-20 colonies from the same bacteria and this is comparing with 1<sup>st</sup> group which was not chitosan treated and this is referred to that treated groups with chitosan before giving bacteria or drenching chitosan with bacteria (*S.Typhimurium*) at the same time, both method effect on the growth and multiplication of bacteria and this agreed with (12,13) who were established that, pretreated with chitosan exhibited resistance to *P.anroginosa* and *L. monocytogenes* infection and demonstrate that chitosan is characterized by high antibacterial effects. (14) revealed decreased number of bacteria in the

caecum, mesenteric lymph node and liver of the mice fed dietary chitosan.

According to (15), chitosan was similarly bactericidal against Gram Positive and Gram Negative organisms indicating non specific action. The key feature of chitosan is depend on its positive charge of the amino group will from poly cationic structure that can interact with the anionic compounds and macromolecular structures of bacteria (16), this charge interaction can alter bacterial surface morphology which either increases membrane permeability, causes leakage of intracellular substances (lactate dehydrogenase, nucleic acid and glucose) or decreases membrane permeability, preventing nutrient transport (17).

The inhibition effect of chitosan show in table (3) was agreed with (18) established wide spectrum of chitosan activity and high killing rate against Gram positive and Gram negative bacteria but lower toxicity toward mammalian cells. In addition, to (13) who performed invitro testing of effectiveness of chitosan against *E.coli*, *P.aeruginosa*, *S.aureus*, *S.paratyphi* and three fungal strains (*C.albicans*, *T.mentagrophytes* and *M.canis*) and demonstrated that chitosan have antibacterial and fungicidal activities, so the differences in inhibitory effects are probably due to variations in experimental materials and conditions; such as the method used, chitosan applied, the medium pH and this agree with (19).

In table (4) there were no significant differences between third and fourth groups and both of them recorded increased of humoral immune response against *S.Typhimurium* in comparing with first group. And the fifth which were do not given chitosan which indicated that drenching of chitosan before infecting with salmonella or at the same time of infection (3<sup>rd</sup> and 4<sup>th</sup> groups respectively) would participated in the elevation of antibody titer that resist the salmonella infection and preventing it from accumulate in the vital

organs for it especially liver and intestine as shown in the table (2), in addition to the postmortum appearance which show heavier weight of immune organs (bursa of fibricius and thymus gland around the neck) in the groups treated with chitosan plus salmonella comparing with not chitosan treated groups (1<sup>st</sup> and 5<sup>th</sup>) so these results suggest improvement in the immune response which agree with (20, 21), so the supplementation with chitosan is expected to increase the availability of circulating amino acids for immunoglobulin synthesis by B lymphocytes which established by (22).

The results here indicate that, the supplementation of chitosan enhanced serum concentrations of immunoglobulines.

Our findings may be caused to that:

1. Binding of chitosan to bacteria (antigen) may initiate the host immune system response.
2. Direct stimulating the host immune response by the active group of chitosan.
3. Competition of chitosan with pathogens for nutrients, thereby improving colonization resistance and protecting the gastro-intestinal tract which agree with (19).

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