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Original Research Article

Immunomodulatory effects of lector on chicks vaccinated with *Mycoplasma gallisepticum* inactivated vaccine

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ABSTRACT

The experiment was designed to investigate the immunomodulating effect of lector 50 on general health and immune response of broiler chicks to *Mycoplasma gallisepticum* vaccination in commercial broiler chicks. the obtained results reveled significantly higher effects on body weight ,bursal, and thymic index on lector treated group of chickens, while no effects on spleen index. Also significant improvement in total and differential leukocytic count as well as significantly higher antibody titer was detected by ELISA in lector 50 treated groups.

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INTRODUCTION

There are large number of immunostimulatory agents that are capable of stimulating the immune response of birds to face the problem of immunosuppression and vaccination failure which constitute a challenge to poultry industry in Egypt and all over the world. The application of immunostimulant is not only to rise the resistance of the birds but also to improve the immune response to vaccination (Afifi,1990 ,Awaad *et al.*, 2000).

Avian mycoplasmas are frequently reported as infectious diseases in poultry. The mostly incriminated species are *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS).

MG is commonly involved in chronic respiratory diseases in chickens and infectious sinusitis in turkeys. Economic losses in broilers usually result from condemnation or down grading of carcasses, reduction in weight gain and increased mortality (Ley, 2003).

This work aims to investigate the immunomodulating effect of lectin on general health and immune response of chicks against *Mycoplasma gallisepticum* vaccine in commercial broiler flocks.

MATERIAL AND METHODS

Materials:

1-Experimental chicks: Two hundred and fifty ,one day- old broiler chicks were obtained from Elahrame company. The chicks were divided into 5 groups and fed on a balanced commercial ration

2-Biochemical kits: *Mycoplasma gallisepticum* antibody test kits were purchased from International Marketing Center (IMC),Egypt and used for estimation of humeral immune response

3- *Mycoplasma gallisepticum* Vaccinal strains: It was obtained from Veterinary Serum and vaccine research institute(VSVRI) ,Abbasia, Cairo.

4-Immunostimulant: *Lector 50*[®]:commercial feed additive product composed of lectine 5000 mg,Xylitol 20000 mg and Fructoligosaccharide 50000 mg,nacl 30000 mg and dist. Water, obtained from microbiotech.USA (patch NO 3355). It was used in drinking water at a rate of 125 cm/ ton/day .

Methods:-

Experimental Design :- Two hundred and fifty, 1-day-old broiler chick were divided into 5 groups as following:

Table (1) showed experimental design

Group NO	NO of birds	Lector Treatment	(M.G)Vaccination at 7 th day of age	Challenge
1	50	1 st -15 day of age	+	All birds were challenged with <i>Mycoplasma gallisepticum</i> strain at 42 day of age 0.5ml /bird intra tracheally
2	50	1 st -7 th day of age	+	
3	50	1 st -7 th day of age	-	
4	50	No	+	
5	50	No	-	

1-Experimental chicks were subjected to vaccination with *Mycoplasma gallisepticum* vaccine and were administrated (for groups 1,2,4 by a dose of 0.5ml and used by sub cutaneous injection at the upper 3rd of birds' neck)

The used *Mycoplasma gallisepticum* vaccine were administrated by S/C rout at 7th day of age

2-Blood samples:-

Blood sample at 1 day of age was taken to ensure freedom from maternal immunity.

Three chicks were chosen randomly from each group at the age of 7-14-21-28-35 day old)

Three blood samples from each group (vaccinated and non vaccinated) were collected with and without anticoagulant weekly for total and differential leukocytic count and for evaluation of humeral immune response by ELISA.

3-Organ collection: Organ samples as spleen, bursa, thymus from each group and control chicks were obtained ,weighted weekly .

4-Bursa/body weight ratio, bursa index and bursa/body weight index

were calculated according to **Sharma et al.(1989)**.

5-challenge test:- birds were challenged with *Mycoplasma gallisepticum* at 42 days old through intratracheal rout using a dose of 0.5 ml of bacterial suspension in sterile saline (1.0*10⁸ CFU/ml) according to **Singab (1987)** and kept under observation for 1 week post inoculation .

6-Evaluation of humeral immune responses by ELISA kits according to instruction of the manufacture

7-Total leukocytes and differential leukocyte count were done according to **Feldman et al. (2000)**.

Results

Effect of lector on body weight (B.W), bursal, Spleen and thymus index on different groups of broiler chicks:

lector treated group(1,2,3) showed significant higher body weight,bursal and thymic index at $p \leq 0.05$ than non-treated group (4,5) at 35th days of age while no significant effects on spleen index as shown in table 2.

Table (2): Effect of lector on body weight (B.W), Bursal, Spleen and thymus index on different groups of broiler chicks

Parameter	Age/day	Group 1	Group 2	Group 3	Group 4	Group 5
Body weight	14	351±12	324±11	339±12	314±10	301±9
	21	693±22	618±22	675±20	585±18	545±18
	28	1116±33	1059±30	1.55 ±30	1003±28	955 ±23
	35	1611±42	1480±40	1410±37	1280±33	1160±30
Bursal index	14	0.613±0.018	0.544±0.014	0.551±0.014	0.410±0.011	0.455±0.011
	21	1.496±0.12	1.344±0.11	1.546±0.11	1.029±0.08	0.926±0.07
	28	1.912±0.14	1.780±0.13	1.870±.13	1.553±0.11	1.360±0.11
	35	2.540±0.18	2.184±0.17	1.911±0.15	1.882±0.15	1.760±0.14
Spleen index	14	0.581±0.018	0.527±0.018	0.505±0.018	0.345±0.016	0.333±0.016
	21	0.794±0.041	0.778±0.041	0.748±0.40	0.624±0.038	0.624±0.038
	28	1.163±0.078	1.170±0.079	1.110±0.07	1.056±0.06	1.086±0.06
	35	1.263±0.087	1.281±0.088	1.210±0.081	1.156±0.080	1.186±0.080
Thymus index	14	2.761±0.08	2.31±0.06	2.33±0.06	2.2±0.05	2.1±0.05
	21	3.7±0.1	3.3±0.09	3.5±0.09	2.9±0.07	2.9±0.07
	28	6.48±0.13	6.15±0.12	6.18±0.12	5.01±0.11	5.07±0.11
	35	7.67±0.18	7.1±0.16	7.1±0.16	5.8±0.13	5.8±0.13

Table (3) Effect of lector on total leukocytic count (TLC)

Group No	Lector treatment	<i>Mycoplasma gallisepticum</i> Vaccination	Total leukocytic count ($10^3/\mu\text{l}$).			
			14 day	21 days	28 days	35 days
1	+	+	10.5±0.19	12±0.29	11.5±0.31	10.5±0.22
2	+	+	10.1±0.22	11.5±0.31	10.1±0.17	9.8±0.2
3	+	-	9.8±0.18	10.5±0.17	8.5±0.17	9±0.18
4	-	+	8.5±0.17	9.1±0.15	9.1±0.14	8.8±0.14
5	-	-	8.5±0.14	8.3±0.13	8.5±0.14	8.2±0.12

Table (4) effect of immunostimulant on differential leukocytic count of *Mycoplasma gallisepticum* vaccinated and non vaccinated broiler chickens

Group No	Lector treatment	M.G Vaccination	Neutrophil				Lymphocytes				Monocytes				Eoosinophil			
			14 Day	21 day	28 day	35 day	14 day	21 day	28 day	35 day	14 day	21 day	28 day	35 day	14 day	21 day	28 day	35 day
1	+	+	36	35	32	32	65	68	73	71	5	5	5	4	3	3	2	2
2	+	+	33	30	28	27	64	63	65	63	5	4	4	4	3	3	2	2
3	-	-	31	30	22	24	62	64	61	60	5	4	4	5	3	3	3	3
4	-	+	31	32	24	23	63	58	56	54	4	5	5	4	3	3	3	3
5		-	30	31	24	23	61	58	56	54	5	4	4	4	3	3	3	3

Effect of lector on optical density measured by ELISA test on different groups of broiler chicks:

Lector Treated group (1,2)and vaccinated with *Mycoplasma gallisepticum* showed significant increase in ELISA Optical density . than non-vaccinated group(3,5) and showed significant increase than vaccinated non treated group (4) as shown in table 5

Table (5) Optical density (O.D) as measured by ELISA test for experimental broiler chicks.

Group No	Lector treatment	<i>Mycoplasma gallisepticum</i> Vaccination	Optical density					
			0 day	7 days	14 days	21 days	28days	35days
1	+	+	0.485	0.380	0.801	1.220	1.432	1.001
2	+	+	0.485	0.380	0.716	1.211	1.322	0.965
3	+	-	0.485	0.380	0.440	0.405	0.365	0.410
4	-	+	0.485	0.380	0.575	0.827	0.940	0.801
5	-	-	0.485	0.380	0.405	0.387	0.390	0.378

Protection rate to *Mycoplasma gallisepticum* M.G challenge in experimental broiler chicks

Detection of protection rate to *Mycoplasma gallisepticum* challenge in experimental broiler chicks ,data presented in Table (6) showed high protection rate in treated vaccinated group (1,2) without any clinical signs , while vaccinated non treated group (4) protected with mild respiratory signs. On the other hand treated non vaccinated group(3)show moderate clinical signs as compared with non vaccinated non treated group(5) which showed sever respiratory signs in the form of ralls, difficult breathing and odema in the head as shown in **table 6**

Table (6) Protection rate to *Mycoplasma gallisepticum* (M.G) challenge in experimental broiler chicks

Group No	Lector Treatment	<i>M.G</i> vaccination	No of Bird	No of Dead	Respiratory Signs	%
1	+	+	20	-	-	100%
2	+	+	20	-	-	100%
3	+	-	20	-	+	35%
4	-	+	20	-	+	100%
5	-	-	20	-	+++	0%

Respiratory signs in the form of ralls, odema on the head, difficult breathing

Discussion

Very few studies have focused on the involvement of dialatory lectins in innate immunity, however, as many receptors on mammalian cell surfaces are glycoproteins, some lectins may well be able to bind to PAMPs, expected that lectins have an effect on the innate immunity

Mycoplasma gallisepticum is the causative agent of chronic respiratory disease in chickens characterized by rales, difficult breathing

,swelling in the infraorbital sinus

This work aims to investigate the immunomodulating effect of the used lectin 50 on general health and immune response of broiler chicks to *Mycoplasma gallisepticum* vaccination in commercial broiler chickens. For providing this points, experiment was designed, where two hundred and fifty, one day old broilers chickens were divided into 5 groups (50 for each group) in different cages treated, vaccinated and challenged as Table (1).

For evaluation of the effect of lectin 50 on average body weight of *Mycoplasma gallisepticum* vaccinated and non vaccinated broiler chicks, data presented in Table(2) revealed that lectin treated group(1,2,3) showed significantly higher body weight, bursal, and thymic index at $p \leq 0.05$ than non treated group (4,5) at 35 days of age while showed non significant effects on spleen index. This finding was agreed with (Savage and Zakrewska 1996) who reported that the removal of potential pathogens from the intestinal tract of growing animals may provide a more favorable environment for the digestion, absorption, and metabolism.

For studying the effect of lectin on total leukocytic count (TLC) of *Mycoplasma gallisepticum* vaccinated and non vaccinated broiler chicks Data presented in Table(3) showed significantly higher TLC in lectin treated group (1,2,3),(10.5,9.8 and 9) respectively in front of (8.8 and 8.2) for untreated group (4,5)

Also data presented in Table (4) and revealed the effect of lectin on differential leucocyte count of *Mycoplasma gallisepticum* treated and non treated group, where lectin treated and vaccinated group 1,2 showed higher neutrophil (32,27) in front of lectin treated and not vaccinated (24) and control group (23). also lymphocyte count was higher in group (1,2), (71 and 63) (treated and vaccinated group) than other groups at 35 day of age

For studying the effect of lectin on immune response to vaccination by *Mycoplasma gallisepticum* vaccine, Data presented in Table (5) revealed the results of Optical density as measured by ELISA,

The result showed significantly increased in Optical density of serum samples collected from treated vaccinated groups 1,2 than non treated vaccinated group (4) (1.432, 1.322 and 0.940) respectively

This indicates that the interaction of lectins with the immune system causes B-lymphocyte proliferation which is manifested by immunoglobulin synthesis increasing antibody response

The activation of Antibody response in lectin treated vaccinated groups is due to activation of B-cells located in the lamina propria, which is the last step B-cell maturation and increase Peyer's patches lymphocytes to control the infection.

Stimulation of host immune response and antibody production by lectins comes in agreement with Kjaerup et al. (2014) who showed that addition of MBL to IBV vaccine enhanced the production of IBV-specific IgG antibody production where it induce increasing in the number of circulating CD4, CD8 and T. cells

For detection of protection rate to *Mycoplasma gallisepticum* challenge in experimental broiler chickens, data presented in Table (6) showed high protection rate in treated vaccinated group (1,2) without any clinical signs, while vaccinated non treated group (4) protected with mild respiratory signs. On the other hand treated non vaccinated group (3) show moderate clinical signs as compared with non vaccinated non treated group (5) which showed severe respiratory signs in the form of ralls, difficult breathing and odema in the head Fig(8)

From previous results we could concluded that, lectins has potential immunomodulatory influence, where lectins may directly or indirectly affect the immune response by binding to surface glycanse on gut epithelial cells, lectin may cause increased endocytosis and increased gut permeability, these effect may results in increased absorption.

From previous discussed data we could recommend the use of lector50 as immunostimulant to improve general health condition and body weight gain in commercial broiler chicks. As well as to improve the immune response to vaccination

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"التأثير المناعي لمادة الليكتور في الكتاكيت المحصنة باللقاح المثبط لبكتريا الميكوبلازما جاليسبتيكم"

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الملخص العربى

تم تصميم تجربة لدراسة مادة الليكتور على الصحة العامة ورد الفعل المناعى فى كتاكيت بدارى التسمين المحصنة بلقاح الميكوبلازما جاليسبتيكم وأظهرت النتائج زيادة فى كل من وزن الجسم ، الغدة التيموسية ، غدة فابريشيس والطحال فى المعاملة بمادة الليكتور أيضا هناك تحسن فى العدد الكلى والنوعى لكرات الدم البيضاء وزيادة فى الاجسام المناعية عند القياس باختبار الإليزا.