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Original Research Article

Factors affecting the immunogenicity of *E. coli* O_{78} vaccine in chickens

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ABSTRACT

Bacterial disease still has serious problem in the intensive poultry production. In the recent years, particular concern has been raised by high incidence of poultry infections by *E. coli*. Analyses of antibacterial properties of essential oils have been carried out by range of researches.

This experiment aimed to study the effect of immunomodulators on the immunogenicity of vaccine *E. coli* O_{78}. In this study 250 broiler chickens were used. They were divided into 5 separated groups all groups vaccinated with *E. coli* O_{78} cebel coarse spray vaccine, except control group, 2 groups treated with immunomodulators, 3 groups challenged with untyped *E. coli* strain, all chickens housed in separated anavar. First group was control, 2nd group was vaccinated only, 3rd group was vaccinated and challenged, 4th group was vaccinated and received immunomodulators and 5th group was vaccinated, received immunomodulators and challenged.

All chickens were observed daily food consumption, weight gained mortality rate, lesion, bioavailability, and weekly collected blood samples from 2-5 birds. The results were summarized as follows; immunomodulators have positive effect on B.W.G, decreased mortality and morbidity rate. The challenge enhanced the effect of *E. coli* O_{78} vaccine and there was marked improvement in bioavailability, B.W.G and immune defense against bacterial and respiratory diseases. Also, immunomodulators increased immunogenicity against bacterial disease through enhancing immune response system, and had synergistic effect with vaccination against *E. coli*.

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Introduction

Infection with Avian pathogenic *Escherichia coli* (*APEC*) is responsible for considerable economic losses in the poultry industry worldwide and is often the most frequently cause of carcass condemnation at processing (Yogarantam, 1995 and Barnes *et al.*, 2008)

E. coli is consider one of the most ordinary microorganisms that affect both animals and humans in a large varieties of diseases, ranging from opportunistic wound infection to severe systemic infections (Gyles,Fairbrother,2010).

APEC is the main source of Colibacillosis in poultry (Solà- Ginés *et al*., 2012). It is the common worldwide disease in poultry flocks especially in the intensive farming system (Chansiripornchai, 2009).

APEC is the most common bacteria isolated from infected yolk sacs (Rosario Cortes *et al*., 2004).

The most prevalent serotypes were detected from 200 broiler chickens are *E. coli*, strains (O_{78}, O2, and O_1) (Gamal *et al*., 2017).

Many of vaccines and vaccination method have been developed including passive and active immunization, use of inactivated and live products, recombinant and subunit vaccines and immunization against specific virulence factors

(Roland et al., 2004; Lynne etal., 2006; Shane, 2009; Yaguchi et al., 2009)

Use of a liposomal inactivated vaccine given by either eye drop or coarse spray stimulated humeral and mucosal antibodies. The number of bacteria in blood was markedly decreased and clinical signs were less sever in vaccinated birds following *APEC* challenge (Yaguchi *et al*., 2009).

Following vaccination there was a decreased mortality and carcass contamination at slaughter, increase average body weight in comparison to previous farm history (Emery et al., 2000)

Vaccinated chickens had less mortalities and lower lesion scores as compared to unvaccinated control groups (Lynne *et al*., 2012).

The vaccine provides protection through cell-mediated immunity rather than circulating antibodies, the vaccine also had a unique drawback as it significantly reduced the weight gain of immunized broilers compared to controls (Filho et al., 2013)

Several essential oil components induce antimicrobial action, some more strongly than others phenols, alcohols, ketones, thymol and aldehydes are mainly associated with the antibacterial actions, although the exact mechanism of actions, has not been fully understood. The mechanism of action of essential oils depends on their unique mechanism but is instead a cascade of reactions involving the entire bacteria. However, it is accepted that the antimicrobial activity depends on the lipophilic character of the components permeate the cell membranes and mitochondria of the microorganisms and inhibit, among others the membrane bound electron flow and therewith the energy metabolism (Nazzaro et al., 2013).

Essential oils also may be an alternative in fighting pathogenic bacteria that developed resistance to many antibiotics (solorzano_santos and Miranda_novales , 2012;de rapper et. al, 2013).

In production houses; especially in summer, when high temperatures and low humidity result in increase in air dust, these conditions lead to respiratory tract disorders in broiler chickens. The treatment of respiratory disorder must be supported with thyme oil and its main components, thymol

and carvacrol which have expectorant, spasmolytic effect and stimulate the respiratory system (Edris, 2007).

The objective of this study Factors affecting the immunogenicity of *E. coli* O_{78} vaccine in chickens to study the effect of viral vaccines on the immune response to *E. coli* O_{78} vaccine and the effect of immunomodulators on the immune response to the vaccine

2. Material and methods

2.1. Animal samples

250broilers One day old broiler chickens were randomly divided into 5 groups of 50 each during the period from April to June 2018; Spring and Summer season the Aniba agriculture secondary school, Animal and chicken production Department Nasr El-Noba center, Aswan, Egypt.

Five chick flocks (one day old Saso broiler chicks) obtained from private poultry company and divided into 50 chicks pre flock chicks were fed commercial ration and water and libitum

2.2 sample collection:

Three chicks were randomly selected from each group (total of 15 chicks at 7, 14,21,28,35 and 42 days of age slaughtered for collection of seka and observation of any clinical signs of diseases and serum samples were separated and preserved in sterile tubes at -20 c till the end of the experimental period .

2.3. Vaccination:

2.3.1. Vaccines (MDS Company)

All flocks under study as well as control group were vaccinated against New castle disease using Hitchner B1 at 6 days of age and Lasota at16 days of age. Infectious bursal disease vaccine was administered 13 days of age. All vaccines were applied in drinking water.

2.3.2. *E. coli* vaccine:

E. coli O_{78} Cebel vaccine (SCBL1000, Nisseiken Co., Japan) was used.

2.4. Experimental challenge with E. coli O_{78} local isolates:

E. coli serogroup O_{78} isolated from cases of pericarditis and completely identified biochemically was obtained from department of Bacteriology mycology and Immunology, Faculty of veterinary Medicine, Beni-Suef University. It was used for experimental challenge in groups under study.

An overnight Mueller Hinton broth culture of *E. coli* was matched with McFarland tube No.0.5 equivalent to $1.5x10^9$ cfu/ml and 0.5 ml of bacterial suspension per bird was installed into the nostrils for 3 successive days in all chicks in group no 2 at the age of 14 days post *E. coli* vaccination.

2.5. Immunomodulators essential oils

2.5. Essential oils:

2.5.1. Carvacolandhydrocinamic acid (Sigma Aldrech Co.):

99% concentration of each oil was used for detection of immunomodulatory effect on chicks under study.

2.6. Immunological Studies:

2.6.1. Interferon-gamma (INF-Y) and Interleukin-6 (IL-6) Kits

ELISA kits (Novatein Bio, Massachusetts, MSA) were used. The micro ELISA plates were pre-coated with antibody specific to chicken INF-Y and IL-6.

2.6.2. Interferon –gamma (INF-Y) assay (Karakolev *et al*., 2013)

INF-Y concentrations were determined using immunoenzymatic assay. In the wells of the ELISA plate, 7 standards were added at a concentrations of 0, 6.25, 12.5, 25, 50, 100 and 200 pg/ ml.

Absorptions were measured at wavelength of 450 nm. Interferon concentrations were calculated from the standard curve by means of software product.

2.6.3. Interleukin-6 (IL-6):

IT was determined using sandwich ELISA where the micro ELISA plate provided has pre-coated with antibody specific to chicken IL-6.

The optical density was measured spectrophotometrically at a wave length of 450 nm. The OD value is proportional to the concentration of IL-6.

The concentration of IL-6 in the samples was calculated by comparing to the OD of the sample to the standard curve according to Kalyuzhny (2005).

3. Results

3.1. Results of performance in control and Experimental groups.

Experimental groups.

Table 1. 1 Results of performance in control and

Group No 1	**1st W**	**2nd W**	**3rd W**	**4th W**	**5th W**	**6th W**
Total No	50(-3)	45(-3)	41(-3)	37(-3)	35(-3)	32(-3)
Total Feed Consumption	3250	4500	10500	11550	14650	26600
Mean Body weight	80	176	390	600	740	1050
Main PM lesions	____	Slight preicraditis	preicraditis	preicraditis		
Mortality rate	0%	10.0%	18.0%	26.0%	30%	36%
Total survived	50(-3)	45(-3)	41(-3)	37(-3)	35(-3)	32(-3)
Group No 2	**1st W**	**2nd W**	**3rd W**	**4th W**	**5th W**	**6th W**
Total No	50(-3)	48(-3)	45(-3)	43(-3)	43(-3)	42(-3)
Total Feed Consumption	3700	5110	10050	11200	16100	28000
Mean Body weight	70	209	382	590	650	1100
Main PM lesions	____	____	preicraditis	preicraditis		
Mortality rate	4%	4%	10.0%	14.0%	14%	
Total survived	48	48	45	43	43	
Group No 3	**1st W**	**2nd W**	**3rd W**	**4th W**	**5th W**	**6th W**
Total No	50(-3)	47(-3)	46(-3)	43(-3)	43(-3)	43(-3)
Total Feed Consumption	343٠	5000	10500	11200	15500	27300
Mean Body weight	80	220	392	600	870	1300
Main PM lesions	____	____	____	____	____	____
Mortality rate	0%	6.0%	8.0%	12%	14%	14%
Total survived	50	47	46	44	43	43
Group No 4	1st W	2nd W	3rd W	4th W	5th W	6th W
Total No	50(-3)	48(-3)	47(-3)	46(-3)	46(-3)	46(-3)
Total Feed Consumption						
Mean Body weight	80	219	416	650	860	1400
Main PM lesions	____	____	____	____	____	____
Mortality rate	0%	4%	6%	8%	8%	8%
Total survived	50	48	47	46	46	46
Group No 5	1st W	2nd W	3rd W	4th W	5th W	6th W
Total No	50	49	49	48	47	47
Total Feed Consumption	3500	4900	9800	11500	14000	26600
Mean Body weight	85	185	400	630	830	1300
Main PM lesions	____	____	____	____	____	____
Mortality rate	0%	2%	2%	4%	6%	6%
Total survived	50	49	49	48	47	47

3.2. Interferon-gamma (INF –Y):

Mean INF-Y concentrations were 72, 120, 230, 190,183 and 173 in the control group compared with 153, 197, 218 , 317, 445 and 367 in the 2nd group and 150, 207 ,463, 647, 683 and 550 in the 3rd group and 152, 193, 507, 637, 690 and 607 in the 4th group and finally 148, 197, 513, 643, 703, and 610 gm/ ml in the 5th group starting from 7 days to 42 days of age.

Table 2. Interferon- gamma (IFN-Y) Table 2

Age	n=	ELISA IFN- gamma Conc. "pg\ml"				
		Gp1	Gp2	Gp3	Gp4	GP5
7 days	**1**	70	120	145	140	150
	2	80	150	140	155	155
	3	80	160	165	160	140
Mean±SD		77±0.344	153±0.534	207±0.360	152±0.344	148±0.444
14 days	**1**	100	180	200	190	210
	2	120	200	230	200	200
	3	110	210	190	190	180
Mean±SD		120±0.244	197±0534	207±0.642	193±0.344	197±0.328

	3	**6**	**21**	**20**	**16**	**18**
Mean ± SD		6 ± 0.15	18± 0.14	18 ± 0.12	14 ± 0.12	17± 0.14
21 days	1	8	18	70	22	24
	2	11	22	90	24	24
	3	10	17	70	23	26
Mean ± SD		10 ± 0.16	19 ± 0.12	77 ± 0.12	23 ± 0.15	25 ± 0.12
28 days	1	10	20	80	27	28
	2	9	23	65	24	29
	3	14	21	55	26	26
		11 ± 0.12	21 ± 0.14	67 ± 0.14	26 ± 0.12	28 ± 0.15
35 days	1	12	18	45	22	23
	2	17	17	40	18	26
	3	13	21	30	25	21
Mean ± SD		14 ± 0.16	19 ± 0.22	38 ± 0.12	22 ± 0.15	23 ± 0.12

3.3. Interleukin-6 (IL-6):

Mean IL-6 in the control group from one week of age till 6 th weeks of age were 2, 6, 10, 11, 14 and 14 pg/ ml compared with the following values in the 2nd group 7, 18, 19, 21, 19 and 19. Meanwhile in 3rd group; gave the following values 8, 18, 77, 67, 38 and 28. The 4th group measures were 6,14, 23 ,26 ,22 and 25 while the 5th group gave 7, 17, 25, 28, 23 and 26 pg/ml.

Table 3. Results of Interleukin-6 (IL6):

Age	n=	ELISA II6 Conc. "pg\ml"				
		Gp1	Gp2	Gp3	Gp4	Gp5
42 days	**1**	12	18	25	22	26
	2	14	18	28	24	27
	3	15	20	30	28	24
Mean± SD		14 ± 0.18	19 ± 0.14	28 ± 0.32	25 ± 0.16	26 ± 0.14
52 days	**1**	13	18	22	20	20
	2	17	19	24	20	25
	3	14	18	26	22	22
Mean± SD		15 ± 0.22	18 ± 0.25	24 ± 0.12	21 ± 0.15	22 ± 0.12

Age	n=	ELISA II6 Conc. "pg\ml"				
		Gp1	Gp2	Gp3	Gp4	Gp5
7 days	**1**	2	7	8	6	7
	2	3	7	7	6	7
	3	2	8	8	7	6
		2 0.12	7 0.15	8 0.12	6 0.14	7 0.12
14 days	**1**	6	15	17	12	15
	2	7	18	18	14	17
	3	6	21	20	16	18
Mean± SD		6 ± 0.15	18± 0.14	18 ± 0.12	14 ± 0.12	17± 0.14
21 days	**1**	8	18	70	22	24
	2	11	22	90	24	24
	3	10	17	70	23	26
Mean± SD		10 ± 0.16	19 ± 0.12	77 ± 0.12	23 ± 0.15	25 ± 0.12
28 days	**1**	10	20	80	27	28
	2	9	23	65	24	29
	3	14	21	55	26	26
		11 ± 0.12	21 ± 0.14	67 ± 0.14	26 ± 0.12	28 ± 0.15
35 days	**1**	12	18	45	22	23
	2	17	17	40	18	26
	3	13	21	30	25	21
Mean± SD		14 ± 0.16	19 ± 0.22	38 ± 0.12	22 ± 0.15	23 ± 0.12

4. Discussion

Concerning weight gain; Reisinger et al. (2012) who noted that B-glucan treated birds have shown significantly higher mean body weight compared with that of the control group in all of examined days. And these results agree with our study which revealed at the end of experiment that G4 (vaccinated mix oil without challenge), most increase in body weight, followed by G5 (vaccinated single oil) which equal with G3 (vaccinated challenged group). G1 (control group) and G2 (vaccinated not challenged) were the lowest degree in body weight gain

Regarding Mortality rates; Mohamed et al. (2011) in study regarded no significant differences in mortalities, average body weights, and lesion scores between the groups vaccinated with the inactivated bacterin and Poulvac *E. coli* when used by coarse spray route. The inactivated bacteria was nearly as effective as the live vaccine in this study which differ from our study which revealed that decreased mortality rates in vaccinated, challenged immune modulated groups (group 5) which was also equal in vaccinated, challenged non immunomodulated groups.

Concerning the effect on immunity by vaccination, challenged and immunomodulators on consequence; Kongkathip et al. (2010) showed that antiviral activity of turmeric essential oil.in recent years studies have been carried out on the use of essential oils in conjunction with vaccination programs, including those against infectious bronchitis, New Castle disease and Gumboro disease.

In another study the results of the experiment show that essential oils promote the production of antibodies ,thus enhancing the efficacy of vaccination (Awaad et al.,2010;Barbour et al.,Faramarzi et al.,2013) and these studies agree with our study which revealed that: First gamma globulin response affected with vaccination and immunodulators.

At the beginning of experiment ,14 days gamma globulin from 210 to 180 in 4 groups, G2 (vaccinated not challenged),G3 (vaccinated challenged group),G4 (vaccinated with mix),G5 (vaccinated with single oil). The G3, G5, G2 showed the highest immune response followed by G4 and G1 (control group). After challenge, G2 showed the lowest immune response. G5 revealed the highest immune response followed by G4 and G3. After challenged, from the 3rd week up to end of experiment, there is remarked increase in the gamma globulin (immune response) observable in G4 followed by G5 and G3.

G1 and G2 showed slightly increase.

Second INL6 immun response on vaccinated and immunodulators of different groups:

Before challenge (1-14 days old) there were no significant difference between the four group G2 (vaccinated not challenged), G3 (vaccinated challenged group), G4 (vaccinated with mix), G5 (vaccinated with single oil). But G1 (control group not vaccinated no immunodulators no challenge) was the lowest result. After challenge G3 (vaccinated challenged without immunodulators), revealed remarkable increase inIL-6 immune response, then other groups (G2,G4,G5) increased, after that gradually decrease happened till the end of experiment.

Also observed that, some similarity between G4 and G5, in the result of IL-6 immune response especially at the end experiment

In another aspect one study revealed that application of essential oils as growth stimulator substitutes in broiler diets does not always improve production performance, and sometimes even makes it worse. This is probably due to a wrong oil concentration (Demir et al.,(2008); Ocak et al., 2008; Brenes and Roura, 2010; Kirkpinar et al., Saleh et al., 2014; Zeng et al., 2015) which differ from our study confirming that correct concentration of essential oils used as immunomodulator in our study.

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