

COMPARATIVE STUDY BETWEEN PHYTOGENIC AND *ESCHERICHIA COLI* VACCINE IN BROILERS

S.M.Abdullah

Sahar.H.Abdulmaged

S.M.Hamad

A. j. Ali

Lecturer

Lecturer

Assist. Lecturer

Assist. Prof

Pathology and Poultry Disease.Coll.Vet.University of Baghdad

E.mail: saharhamdi11@gmail.com

ABSTRACT

The present study carried out to comparative between usage of killed vaccine of *Escherichia coli* with aromatic oils or alone against infection of broiler with pathogenic *E.coli* for this purpose two hundred broiler chicks were collected and randomly divided into four groups. Each group contained fifty chicks, food and drink for all groups ad libitum. The experimental groups were treated as follows: G1 vaccinated with the killed *E. coli* vaccine through subcutaneous injection dose 0.5 ml (3×10^7 CFU/ml) in day five old and received Digestarom® P.E.P sol. in drinking water 6 ml/100L from one day old until end of trial. G2 vaccinated with killed *E. coli* only through subcutaneous injection dose 0.5 ml (3×10^7 CFU/ml) in day five old. G3 received Digestarom® P.E.P sol. in drinking water 6 ml/100L only one day old until end of trial. G4 control negative. The experimental birds in day 25 were challenged intratracheally inoculated with *Escherichia coli* of 0.5 ml was LD₅₀(3×10^8 CFU/ml) in the experiment. Blood samples were taken at day 20 and 35 for assessment of different parameters, mortality and lesions score were determined. The results showed that the use of aromatic oils and received killed vaccine of *E.coli* has a good effect on the immune response, decreasing the mortality rate and lesion score of *E. coli* infected chickens. It can be concluded from the current study that the use of digestarom enhanced the immune response after vaccination with *E.coli* vaccine against pathogenic *E.coli*. It also the use of *E.coli* vaccine with the digestarom in broiler chickens is more effective than using the vaccine alone.

Keywords : Essential oils, haematology tests, colony forming unit, killed vaccine, blood.

عبدالله وأخرون

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دراسة مقارنة بين phytoenic و لقاح *Escherichia coli* في دجاج اللحم

ابتسام جواد علي

سعد محمد حمد

سحر حمدي عبد المجيد

سمير مظهر عبدالله

أستاذ مساعد

مدرس مساعد

مدرس

مدرس

فرع الأمراض وامراض الدواجن كلية الطب البيطري جامعة بغداد

المستخلص

اجريت هذه الدراسة لمقارنة استخدام لقاح *E.coli* المقتول مع الزيوت العطرية او لوحده ضد الإصابة بجراثيم *E.coli* المرضية في دجاج اللحم. ولهذا الغرض تم استخدام 200 فرخ دجاج لحم وقسمت عشوائيا إلى أربع مجاميع. كل مجموعة تحتوي على خمسين فرخ، تم التعامل مع المجموعات التجريبية على النحو التالي: G1 أعطيت لقاح مقتول من ايشريشيا القولون عن طريق الحقن تحت الجلد وبجرعة 0.5 مل (3×10^7 خلية /CFU/ml) في اليوم الخامس واعطيت Digestarom® P.E.P في مياه الشرب 6 مل لكل 100 مل من عمر يوم واحد الى نهاية التجربة و G2 أعطيت لقاح مقتول من *E.coli* عن طريق الحقن تحت الجلد وبجرعة 0.5 مل (3×10^7 خلية /CFU) في اليوم الخامس و G3 أعطيت Digestarom® P.E.P في مياه الشرب 6 مل لكل 100 مل من عمر يوم واحد الى نهاية التجربة اما G4 اعتبرت سيطرة سالبة. وعند عمر 25 يوما تم إصابة جميع المجاميع بجراثيم *E.coli* عن طريق الرغامي وبجرعة 0.5 مل (3×10^7 خلية /CFU) تم جمع عينات الدم عند عمر 20، 35 يوم لاجراء فحوصات الصورة الدموية والمناعية وأظهرت النتائج أن استخدام الزيوت العطرية مع لقاح مقتول من *E.coli* له تأثير جيد على الاستجابة المناعية، وانخفاض معدل الوفيات ودرجة الآفة في الدجاج المصاب بالايشرشيا القولونية. نستنتج من الدراسة الحالية ان استخدام يعزز الاستجابة المناعية للقاح ضد الإصابة بجراثيم *E.coli* المرضية.

الكلمات المفتاحية: زيوت عطرية، فحوصات دموية، مستعمرة لكل وحدة، لقاح مقتول، دم

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INTRODUCTION

Collibacillosis is a common poultry disease caused by *Escherichia coli*, especially the serotype O78 (*E. coli* O78), which causes either an sub-acute infections or acute fatal septicemia (9,42). It has previously been assumed that disease due to *E. coli* is a result of either management insufficiencies, e.g. an inappropriate biosecurity level and insufficient egg hygiene or decreased immune competency of the birds due to concurrent infectious diseases, such as infectious bronchitis or adenovirus (28,32,36). Currently, accumulating evidence has led to the acceptance that certain clones of *E. coli* may act as primary pathogens and cause severe disease and high mortality despite of high standard management, low stress levels and the absence of concurrent diseases (6,12,18,37). The development of the use of herbal or phytogetic plants containing essential oils in the poultry industry has gave successful results (21). Among these, phytobiotics are a species of genus (*Origanum*), aromatic plants of the Lamiaceae family. Several studies have confirmed antimicrobial properties in the volatile oils of *Origanum vulgare* (43 ,44). Hong *et al.*, (25) confirmed the low concentration of ammonia in ileum for broiler chickens, which is well fed to essential oils 125 ppm, which includes essential oils derived from oregano, anise and citrus peel.

MATERIALS AND METHODS

Vaccine preparation: The *E.coli* strain of the O78 serotype grew on the brain heart agar in Roux tubes and incubated at 37 ° C for 24 hours. Bacterial colonies were collected using saline solution, and the bacterial suspension was adjusted to be 3×10^7 CFU / ml. (vaccine dose). The bacteria were then inactivated by adding 0.5% formalin. (Digestarom® P.E.P sol) a commercial phytogetics natural water additive supplement by the Biomin Company, Austria was tested. In this product the main active ingredient is Oregano oil, Anise oil, Citrus oil and Fructo-oligosaccharide (FOS).

Experimental design

Two hundred chicks in one day old (Ross, 308), were brought from local Hatchery–

Baghdad. The chicks were divided randomly into 4 groups each group contains 50 chicks. All groups were vaccinated with live vaccine for Newcastle disease *La Sota* strain with drinking water on day 10 and the booster dose was on day 18, and were vaccinated just once with IBD vaccine at day 13. G1 received killed vaccine of *E. coli* through subcutaneous (S/C) injection dose 0.5 ml (3×10^7 CFU/ml) in day five and received Digestarom® P.E.P sol in drinking water 6cc/100L from one day old until end of trial. G2 received killed vaccine of *E. coli* through subcutaneous (S/C) injection dose 0.5 ml (3×10^7 CFU/ml) in day five old. G3 control positive received Digestarom P.E.P sol in drinking water 6 ml/100L from one day old until end of trial. G4 control negative. The experimental birds in day 25 were challenged intratracheally the bacterial suspension of 0.5 ml was (3×10^8 CFU/ml) in the experiment. Total Red blood cells counting cell/ mm⁶ (35). Total White Blood cell counting cell/ mm³ (37). Packed cell volume (PCV) Reading was taken by using a micro-hematocrit reader according to the method mentioned by (8). Determination of Hemoglobin Concentrations (Hb) The result was expressed using mg / dl. The previous procedure was matched with the Hellige hemometer method described previously by Lamberg and Rothstein (29). Measured the total protein in blood serum Bayourat (Biuret method) was used at commercial kit (RANDOX)[®] was used As mentioned by Henry *et al.*, (23). Bromocresol Green was used to measure albumin levels in the serum by using the commercial kit (TC)[®] produced by the company referred by Doumas and Biggs (11). After obtaining the total concentration of protein and albumin, the following equation is applied for the treatment of serum globulin: Concentration of globulin gm / dl = Total concentration of protein - albumin concentration. The characteristic lesion scoring of organ at postmortem examination the gross lesions of airsacculitis, pericarditis and perihepatitis were scored macroscopically. The lesions score were recorded as described (7) and (17).

RESULTS AND DISCUSSION

The results in(Table 1) shows the Haematological (WBC, RBC, PCV and Hb) of all groups. The most significant difference ($P \leq 0.05$) was found in the group one as compared with another groups separately. After challenge with APEC at 25 days old chicks, there was more leukocytosis in G1,G2 and G3 as compared with the control group. This finding agrees with some researchers (34,30). They found that nitric oxid (NO) also reduces or inhibits the concurrent local LPS-stimulated release of platelet-activating factor (PAF), endothelin-1 (ET-1), thromboxane A2 (TxA2), and 5-hydroxytryptamine (5HT), so decrease tissue damage and vasoconstriction may be due to leukocyte activation. Haematological studies were agreement with Vikash *et al.*, (46) witch revealed macrocytic normochromic anaemia in infected groups. The intensity of anaemia was less in neem leaf extract (NLE) supplemented *E. coli* infected chicks,addition of 10% NLE in drinking water showed improvement in anemia and significant leucocytosis induced by *E. coli* infection. Ryzner *et al.* (40), The decrease in haematological parameters in control negative may be due to break down of erythrocytes by hemolytic enzymes produced by *E.coli* (9) or due to inappetence and diarrhoea leading to nutritional deficiencies and this lead to decrease in number of erythrocytes which then lead to decrease in per cent PCV and haemoglobin concentration (15). Phytogetic used to increase the positive effect of immunity, blood parameter, and physical performance that represent the natural growth and healthy state (21,39,4). Hernandez *et al.* (24) confirmed that Oregano possesses antioxidant bio-properties that enhance the vital functions of the kidney and liver(14). Phytogetic increased production such as acute phase proteins as interleukin (IL)-1 and IL-6, tumor necrosis factor (TNF- α) -like substances. Cachectins are produced by such as extra vascular effector cells as macrophages in response to invasive stimuli. (27,2,3).Table (2) Results of total protein, albumin, Globulin Compared to the challenge control group, the lowest albumin globulin ratio was recorded in

the treated groups. This means a good indicator of high levels of globulin, which later gives a good immunostimulation (31). Thus, chronic antigen stimulation leads to production high amounts of globulin by plasma cells (8). A rise in immunoglobulin may occur (31), Significant increase globulin in vaccinated groups these finding confirmed with(1) mentioned that,the presence of calcium, glycoprotein mannose-binding lectin (MBL) recognizes on the surface of microorganisms. MBL kills microbes by acting as an opsonin or by activating the lectin complement pathway It can be concluded from the current study that the use *E.coli* vaccine with the mixture of essential oils in broiler chickens is more effective than using the vaccine alone. Also the lesion score showed significant difference between different experimental groups (Table 3), the lowest effect in group one compared with other groups and control negative. This agree with Cho *et al.* (10) found that feeding using phytogetic feed additives (PFA) containing essential oils such as thyme and anise which is an effective ingredient in inhibition of *C. perfringens* and *E.coli* in the small and large intestines in broiler chicken. While the significant decrease in mean of score lesion in the vaccinated groups agrees with Hanan *et al.*, (20) reported that vaccinated with inactive *E. coli* by formalin protected the vaccinated bird from challenge with APEC at 3 weeks of age and slightly recorded pathomorphological changes. Concerning the mortality rate(Table 4), it was observed that group 1 showed 10% of chickens, group 2 showed 16% and group 3 showed 20% mortality compared with control group witch 60%. Hong *et al.* (25) used 125 ppm of essential oils (Oregano, anise and citrus peel powder) to feed broiler chickens, noting a significant increase in survival rate of 10%. The results were consistent with Gbenga *et al.* (16) demonstrating that using garlic supplements to feed broiler chickens helps reduce mortality by 1.67-3.33%. This explains the effective role of essential oils extracted from garlic in increasing plant digestibility and reducing the presence of *E .coli* and clostridium sp. in intestinal content. As

confirmed by Jamoroz *et al.* (26), the use of 80% garlic in broiler chicken feeding helps to inhibit the growth of *E. coli*, Salmonella and Staphylococcus in the contents of the

intestines, thus reduces mortality rate due to biological cleaning of the intestinal surface of all birds

Table 1. Results of Hematological parameters to different types of vaccine of the experiment (Mean ± SE).

Group	WBC (x10 ³)		RBC (x10 ⁶)		PCV (%)		Hb (gm/dl)	
	20 day	35 day	20 day	35 day	20 day	35 day	20 day	35 day
G1	16.84±0.35 A	20.92±0.08 A	2.01±0.03 A	1.75±0.05 A	38.63±0.17 A	47.48±0.25 A	10.2±0.33 A	11.58±0.31 A
	14.8±0.27 B	18.15±0.11 B	2.02±0.03 A	1.53±0.02 B	37.57±0.17 A	45.67±0.26 B	9.6±0.27 AB	10.7±0.24 B
G2	13.1±0.42 C	16.05±0.13 C	2.03±0.02 A	1.42±0.01 C	37.01±0.12 C	44.35±0.17 C	9.3±0.14 B	10.32±0.17 B
	8.1±0.44 D	12.7±0.18 D	2.01±0.04 B	1.07±0.04 D	36.74±0.42 C	19.94±0.35 D	9.8±0.26 AB	6.57±0.14 C
G3	4.465±0.006 A	4.478±0.015 A	2.650±0.003 A	1.862±0.008 A	1.810±0.004 B	2.668±0.024 A	1.46±0.022 A	0.72±0.033 B
	4.122±0.003 C	4.238±0.005 B	2.411±0.012 B	1.754±0.004 B	1.710±0.003 C	2.483±0.012 B	1.40±0.005 B	0.70±0.011 C
G4	4.215±0.003 B	4.025±0.003 C	2.378±0.003 C	1.597±0.003 C	1.835±0.003 B	2.428±0.003 C	1.29±0.003 C	0.65±0.004 D
	4.021±0.007 D	3.662±0.006 D	2.119±0.014 D	1.587±0.012 C	1.902±0.005 A	2.075±0.008 D	1.11±0.014 D	0.76±0.016 A

The capital letters indicate a significant difference at level (P <0.05) between the averages within the columns

Table 2. Results of total protein, albumin, globulin (Mean ± SE) and albumin globulin ratio.

G	Total protein (gm/dl)		Albumin (gm/dl)		Globulin (gm/dl)		A/G ratio (%)	
	20 day	35 day	20 day	35 day	20 day	35 day	20 day	35 day
G1	4.465±0.006 A	4.478±0.015 A	2.650±0.003 A	1.862±0.008 A	1.810±0.004 B	2.668±0.024 A	1.46±0.022 A	0.72±0.033 B
	4.122±0.003 C	4.238±0.005 B	2.411±0.012 B	1.754±0.004 B	1.710±0.003 C	2.483±0.012 B	1.40±0.005 B	0.70±0.011 C
G2	4.215±0.003 B	4.025±0.003 C	2.378±0.003 C	1.597±0.003 C	1.835±0.003 B	2.428±0.003 C	1.29±0.003 C	0.65±0.004 D
	4.021±0.007 D	3.662±0.006 D	2.119±0.014 D	1.587±0.012 C	1.902±0.005 A	2.075±0.008 D	1.11±0.014 D	0.76±0.016 A

*The capital letters indicate a significant difference at level (P <0.05) between the averages within the columns

Table 3. Pathological changes in thoracic air sac, heart and liver after infection with APEC strain.

Group	Air sac score	Heart score	Liver score
G1	C 0.5±0.03	C 0.25±0.18	C 0.35±0.07
G2	B 0.9±0.06	B 0.8±0.24	B 0.75±0.13
G3	B 0.75±0.04	B 1±0.13	B 0.9±0.14
G4	A 1.25±0.02	A 1.8±0.05	A 1.75±0.51

*The capital letters indicate a significant difference at level (P <0.05) between the averages within the columns

Table 4. Protection test Development of clinical signs and mortalities post challenge with APEC of the experiment

	G1	G2	G3	G4
Sign %	10	20	30	80
Mortality %	10	16	20	60

Clinical signs = That include one or more dropping, respiratory signs and swollen head

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