Effect of injection the broiler hatching eggs with vitamin E and cod liver oil on some their productive traits and immune response to Newcastle disease vaccine

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Summary

Four hundred fertile eggs of broiler breeder Ross strain were incubated in commercial Hatchery. Eggs were injected into amniotic fluid on 18th day of incubation after divided into four treatment groups (100 eggs per treatment) with two replicates. First treatment was injected 100 µl of PBS, second treatment was injected with100 µl of inactivated ND vaccine, the third treatment was injected with 100 µl of inactivated ND vaccine and 50 µl vitamin E (oily form) and finally the fourth treatment was injected with 100 µl of inactivated ND vaccine and 50 µl Cod liver oil. All injected eggs were carried back into hatchery for complete hatching process. Hatched chicks were transported to the farm of the Veterinary Medicine College/University of Baghdad for 42 days from 6/10/2011 to 16/11/2011. The hatched chicks from the previously treated groups were distributed into four treatments with two replicates for up to the experimental end. Results of treated groups showed significant ($P \leq 0.05$) increase in body weight, weight gain, as well as significant reduce in feed intake and improvement in feed conversion ratio, also, antibody titers against Newcastle disease virus of treated groups showed significant ($P \leq 0.05$) increase progressively with age until the end of the experiment compared with broiler chicks of control.

Keywords: In ovo injection, Broiler, Vitamin E, Cod liver oil, Immune response.

Introduction

Many of researches were showed possibility early nutrition of chicken embryos (known as in ovo feeding), it was very important to reduce exposure of nutritional diseases (less supply of energy, less supply of some vitamins and minerals), as well as this technique has proven requirements for embryos of modern broiler breeds more than old breeds (1). The additional energy source supported the late-term broiler embryos and improve early growth to enhance the genetic potential for late embryonic and early post hatch growth (2) and help chicks to enhance ability of digestion and metabolism after hatching which led to improve the productive traits and accelerate access to marketing weight (3). Poly unsaturated fatty acids which are found in cod liver oil, richest food omega- 3 are important in growth and development of broilers, as well as, elevation in the nutritional value of poultry meat promotion ratio, they played best role in the prevention of heart disease, atherosclerosis, high blood pressure, arthritis, and other types of infections, in addition to increase the immunity and reduce the incidence of cancerous diseases in humans and animals. Improvement in immune function of broilers fed with omega-3 poly unsaturated fatty acids (4-6).

In poultry, vaccines are generally administered as aerosols, oculo-nasal drops and through drinking water or by injection , Recently, in ovo vaccination technique has been developed that several advantages like neonatal resistance, administration of vaccine in large number of eggs with standard time and soon after hatching birds are protected against the disease, this way consider modern technologies and practically of regarding economy, and it was used to give several types of vaccines for different viral diseases. Embryos could be vaccinated against Newcastle disease on day 18 of incubation, newly hatching chicks have gave early protection despite presence of maternal antibodies (7 and 8).

Vitamin (E) one of the main types of vitamins that have a role in the vitality of the body and health of the bird, vitamin (E) increased the level of immune response and protect the body from the stress (9), and stimulated the immune system as an active

natural antioxidant in the body (10). It worked to prevent the damages of body cells by prevent formation of free radicals and the oxidation of unsaturated fatty acids to maintain the membranes of lymphocytes and macrophages cell responsible for stimulation cellular and humoral immunity and gave body the ability to resist different bacterial infection (11). The objective of this study is to determine the effects of injection of broiler hatching egg with vitamin E and cod liver oil on some their productive traits and immune response to Newcastle disease vaccine.

Materials and Methods

A total of 400 fertile eggs of broiler breeder Ross (308) were incubated in commercial hatchery (Al-Saud Hatchery/Al-Husseinia-Karbala) after grading (culled the abnormal eggs and misshapen). All eggs were distributed into four treatments, 100 fertile eggs were assigned to each treatment with two replicates per treatment. The fertile eggs had been injected at 18 days of incubation before transporting the eggs from Setter to the Hatcher according to (12). First treatment kept as control was injected with 100 µl of Phosphate Buffer Saline (PBS), second treatment was injected with 100 µl of inactivated ND vaccine (Clone 30 ≥ 50 PD_{50}), third treatment was injected with 100 µl of inactivated ND vaccine and 50 µl vitamin E (oily form) and finally fourth treatment was injected with 100 µl of inactivated ND vaccine and 50 µl Cod liver oil.

In ovo procedure, after embryonic inspection by candling with vitality checking and determined the air space for injection, then in ovo amniotic injection were carried out under sterile place by using automatic syringe with needles of length at 2.5 cm, gauge needle 23, the entire length of the needle was extended into the hatching egg to ensure that the needle punctured the amnion, immediately after the injection, the site was sealed with sterile paraffin wax and eggs were returned to the incubator (13). Then, newly hatching chicks had been transported to the farm of the Veterinary Medicine College, University of Baghdad. The hatched chicks from the previously treated groups were distributed to four treatments (50 chicks were selected randomly from each treatment with two replicates).

The hatched chicks from the previously treated groups were weighed to determine the initial body weight, then distributed into four treatments with two replicates up to end of the experimental. Farm ground was provided with suitable litter and other factors like temperature, lighting and ventilation were controlled. Birds were offered feed and water *ad libitum*. All birds received common feeds (Table, 1) from placement until the end of experiment. Nutritional requirements were adjusted according to the Nutritional Requirements Council (14).

Birds were weighed weekly by sensitive balance until 6th week of age. Body weight gain, feed intake and feed conversion ratio were calculated at the same time. Mortality were recorded daily. Chicks of control group were vaccinated with Newcastle disease vaccine (Dose 10^8 EID_{50}/ml attenuated ND Clone 30) at (10, 20 and 30) days-old, while other treatments were left without vaccination after hatching process. The composition of experimental diet is shown in (Table, 1).

Table, 1: Composition of experimental diet prepared in this study

Ingredients	Percentages of ingredients in Starter (1-21 days)	Percentages of ingredients in finisher (22-42 days)
Yellow corn	44.00	46.00
Soybean meal (45% crude protein)	24.50	21.00
Wheat	19.00	19.00
Protein concentration*	10.00	10.00
Sunflower oil	1.50	3.00
Dicalecium phosphate	0.70	0.70
Salt (Nacl)	0.30	0.30
Total	100	100
Calculated chemical analysis		
Crude protein %	21.45	20.06
Metabolizable Energy (kcal / kg)	2973	3091
Calcium (%)	0.95	0.95
Available phosphorus (%)	0.66	0.66
Methionine (%)	0.72	0.71
Lysine (%)	1.15	1.10

* Protein concentration type (Provemi) Jordan made. Chemical contain : Protein 40% , M.E 2250 Kcal /Kg, Fat 6.5%, Fiber 3.5%, Ash 31.0% ,Calcium7.0%,Avialable phosphorus 3.3%, Methionine 1.80%, Lysine 3.0%, Meth.+Cystine 2.3%, Sodium 1.3%,Chloride 2.3%.

All vaccination were carried out via drinking water. The same vaccine strain (ND Clone 30 ≥ 50 PD_{50} Intervet-Holland) was used in ovo vaccination. Preparation of the watering system to be used through removal of all disinfectants, such as chlorine was done two days prior to vaccination. It was best to buffer the system by flushing it with a weak solution of powdered skim milk, generally 4 gm powdered skim milk to one liter of water (15). Blood samples were withdrawn from wing vein by disposable syringe, then blood samples were centrifuged at (2300 rpm) for 5 minutes as recommended (16). Samples from these birds were collected at 9, 19, 29 and 39 days of age to detect Newcastle disease virus antibody titers using Enzyme Linked Immunosorbent Assay (ELISA).

All data of this study were analyzed statistically by using analysis of variance (ANOVA) and least significant differences (LSD) were used to differentiate among mean of results (17).

Results and Discussion

The highest mean body weight and body weight gain of the third and fourth treatment groups were significantly ($P \leq 0.05$) increased as compared with the second and control groups on 3^{th}, 4^{th}, 5^{th} and 6^{th} week (table 2). Also, the results according to table (2) showed the second, third and fourth treatments on 3^{th} and 4^{th} week a significant ($P \leq 0.05$) increase in feed intake compared with control. As well as, the same treatment (third and forth) showed a significant ($P \leq 0.05$) improvement in feed conversion ratio on 3^{th}, 4^{th}, 5^{th} and 6^{th} week of birds age compared with second group and control.

Results demonstrated that using inoculation with vitamin E had a significant role on growth and health of chicks because the protective role of vitamin E on the cell membranes of hepatocytes from oxidative damage which happen with free radicals action by inducing lipid peroxidation of polyunsaturated fatty acids in the cell membrane, resulting in abnormal membrane integrity, therefore, inversely affects the metabolic activity of hepatocytes associated with a reduction in synthesis of proteins or metabolism of carbohydrates and fats, after that, glycogen storage may be affected, as well as, vitamin E was activated liver hepatocytes to produce protein which caused elevation body weight (18).

In addition to, vitamin E was accomplished to maintain the cells in all body specialized cells of gastrointestinal tract increased intestinal activity of chick that led to improve of utilization all diet ingredients for body structures (19). Also, the results revealed a significant increase of the mean body weights of the fourth treatment in comparing with second and control groups and no significant ($P > 0.05$) differences with the third group (Table, 2), Also, omega-3 fatty acids have importance in the growth and development of body cells because it was entered in composition of cell membranes of cells. Therefore, digestibility and absorption of nutritional elements were enhanced and increasing digestive enzyme activity (2).

These results might be reflected the ability of chicks to control their feed intake according to the extent utilization of the diet. The results of this study showed that there were a greater reduction in feed conversion ratio of treated chicks with vitamin E as compared with other treatments and control group (Table 2). These results demonstrated the role of vitamin E in preserving gastrointestinal tract tissues and then enhanced intestinal development that led to increase intestinal capacity for good absorption, also the same results showed an increase in bird ability for utilization of feed ingredients which was reflected by increasing in body weight of those treated groups, consequently, vitamin E has demonstrated to be an antioxidant that was scavenged the free radical generated by metabolic processes inside the body. This observation was agreed with (20-24) that vitamin E has positive effect on growth and body weight because vitamin E acts as a coenzyme in cellular membranes and as a scavenger for free radicals that might be destructive to membrane and internal cellular component.

Table, 2: Mean ± Standard Error body weight, body weight gain, feed intake and feed conversion ratio for different groups.

Groups		Group 1 (Control) Inject with sterile PBS	Group 2 Inject with ND vaccine	Group 3 Inject with ND vaccine and vitamin E	Group 4 Inject with ND vaccine and cod liver oil
Variable	Age (week)				
Body weight (gm)	1st wk	105±1.78	115.5±1.36	129.8±1.18	130.7±0.64
	2nd wk	317.8±4.97	329.4±5.63	371.5±9.02	328±6.41
	3rd wk	533.1±30.41 c	594.5±6.83 b	687.2±9.69 a	642.8±5.22 a
	4th wk	1081.4±21.12 d	1182.8±25.23 c	1362.1±18.83 a	1284.2±13.2 b
	5th wk	1482.1±4.40 d	1646.4±35.28 c	1880.7±18.31 a	1806.4±8.03 a
	6th wk	1900±15.11 d	2169.2±144.8 c	2492.8±12.76 a	2386.7±13.89 b
Body weight gain (gm)	1st wk	60.8±1.69	74±1.39	82.7±1.49	82.7±0.74
	2nd wk	212.8±5.56	212.7±6.49	241.7±9.75	230.2±6.16
	3rd wk	243.8±13.46 b	307±15.53 a	317.1±9.62 a	314.8±10.01 a
	4th wk	519.7±22.25 c	588.2±23.30 b	674.8±23.25 a	641.4±12.21 a
	5th wk	389.2±24.45 c	463.5±25.25 b	521.4±2.45 a	522.1±6.49 a
	6th wk	417.8±12.94 b	380±28.01 c	609.2±11.42 a	580.2±13.21 a
Feed intake (gm)	1st wk	119.6±14.9	140.7±13.5	152.9±6.8	148.6±22
	2nd wk	350.8±4.6	328.7±12.8	369.7±7.6	230±11.3
	3rd wk	388.9±9.8 b	427.8±28 a	456.3±3 a	459.3±19.1 a
	4th wk	801.9±39.3 b	843.1±18.5 ab	892.8±2.5 a	851.6±5.2 ab
	5th wk	921.2±1.2 b	1031.5±54.8 a	900.3±9.8 b	924.8±23 b
	6th wk	950.6±51 b	870.2±70.9 c	990.2±15.4 ab	1010.5±10.5 a
Feed conversion ratio	1st wk	1.976±0.2 a	1.902±0.1 a	1.849±0.08 b	1.796±0.02 b
	2nd wk	1.647±0.02 a	1.556±0.06 b	1.529±0.03 b	1.622±0.05 ab
	3rd wk	1.594±0.04 a	1.612±0.1 a	1.445±0.01 b	1.454±0.06 b
	4th wk	1.543±0.07 a	1.433±0.03 b	1.322±0.003 c	1.327±0.008 c
	5th wk	2.366±0.003 a	2.224±0.1 a	1.726±0.01 b	1.771±0.04 b
	6th wk	2.274±0.1 a	2.318±0.1 a	1.625±0.02 c	1.741±0.01 b

Different small letter horizontally refer to significant differences at level (P ≤ 0.05) among mean of groups.

Results of (table, 3) showed a significant differences in antibody titers against Newcastle disease virus measured by ELISA (Optical Density Value) at level (P ≤ 0.05) among the treatments and control group at (9, 19 , 29 and 39) days old.

Table, 3: Mean ± standard error in mean of antibody titers against Newcastle Disease Virus measured by ELISA (Optical Density Value) for different groups.

Group Age	Group 1 (Control) Inject with sterile PBS	Group 2 Inject with ND vaccine	Group 3 Inject with ND vaccine and vitamin E	Group 4 Inject with ND vaccine and cod liver oil
9 d	0.403±0.007 c	0.414±0.004 B	0.429±0.009 a	0.430±0.010 a
19 d	0.422±0.007 d	0.468±0.003 C	0.508±0.003 a	0.495±0.005 b
29 d	0.495±0.005 d	0.588±0.005 C	0.690±0.001 a	0.644±0.001 b
39 d	0.547±0.008 D	0.681±0.008 C	0.801±0.001 a	0.779±0.002 b

Different small letter horizontally refer to significant differences at level (P ≤ 0.05) among mean of groups .

Data showed significantly (P ≤ 0.05) higher antibody titers in blood serum samples of the third treatment than the second, fourth treatments and control at 9, 19, 29 and 39 days old. Despite, there was no significant (P > 0.05) differences between the third and fourth treatments at 9 days old, the fourth treatment showed a significant increase in antibody titers

as compared with the second treatment and control at 9,19,29 and 39 days old. On the other hand, the second treatment recorded a significant ($P \leq 0.05$) increase in antibody titers as compared with the control group at different periods.

Results of (Table, 3) demonstrated the effect of vitamin E in increase of immune response by activation and division B-Lymphocyte led to increase antibody titers as indicated that defense of broiler chicks against Newcastle disease virus. This was agreed with (25-27) who confirmed that estimation antibody titers in birds serum gave a good evidence to appointment immunity of birds against Newcastle disease virus, also, these positive results indicated that effect of vitamin E in preservation of the immune cells by inhibit free radicals formation as product due to cellular immune response during phagocytosis process against micro organisms. Furthermore, vitamin E kept cellular membranes flexibility which have achieve role in antigen diagnosis, this was agreed with (28) who mentioned that immunomodulatory effect of vitamin E on CD4 and CD8 - T cells, consequently, improved cell mediated immune response in birds body when male broilers fed diets with various levels of vitamin E supplementation. However, results of the fourth treatment showed a significant increase in antibody titers as compared with the second treatment and control, these finding were agreed with (5) who explained that role of omega-3 fatty acids as immunity stimulators of cytokines and help B-lymphocytes to increase antibodies production. Cod liver oil richest with long chain omega-3 fatty acids (EPA and DHA) could be cause an efficient improvement in broiler immunity and the antibody production of broilers (29 and 30).

According to the results were obtained from this study it can be concluded that chick embryos injection on 18th day of incubation with vitamin E and cod liver oil improved body weight, body weight gain and improvement in feed conversion ratio, enhanced immune response of birds against Newcastle disease vaccine.

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تاثير حقن بيض تفقيس فروج اللحم بفيتامين E وزيت كبد الحوت في بعض الصفات الانتاجية والاستجابة المناعية للقاح مرض نيو كاسل

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الخلاصة

استخدمت 400 بيضة مخصبة من سلالة روز وحضنت في احد المفاقس التجارية , تم حقن البيض المخصب في كيس الامنيون بعمر 18 يوم من الحضن بعد تقسيمه الى اربعة معاملات (100بيضة /معاملة) وبواقع مكررين لكل معاملة. المعاملة الاولى حقنت 100 مايكروليترمن محلول دارئ الفوسفات المعقم كمجموعة سيطرة. المعاملة الثانية حقنت 100 مايكروليترمن لقاح الزيتي المبطل لمرض النيوكاسل. المعاملة الثالثة حقنت 100 مايكروليترمن لقاح الزيتي المبطل لمرض النيوكاسل و50 مايكروليتر من فيتامين (E) . المعاملة الرابعة حقنت 100 مايكروليترمن لقاح الزيتي المبطل لمرض النيوكاسل و50 مايكروليتر من زيت كبد الحوت. وبعد الانتهاء من عملية الحقن تم ارجاع البيض المحقون إلى المفقسة لغرض اكمال عملية التفقيس.الافراخ الفاقسة نقلت إلى قاعة التربية في حقل الدواجن التابع لكلية الطب البيطري / جامعة بغداد لاكمال التجربة والتي استمرت لمدة 42 يوما للمدة من 2011/10/6 ولغاية 2011/11/16 حيث تم توزيع الافراخ حسب معاملاتها السابقة لغرض دراسة تاثير المواد المحقونة سلفا في الصفات الانتاجية والمناعية لفروج اللحم .اظهرت النتائج تفوق المجاميع المعاملة معنويا ($P \leq 0.05$) في الاداء الانتاجي لفروج اللحم المتمثل بوزن الجسم الحي ، معدل الزيادة الوزنية الأسبوعية ، استهلاك العلف الأسبوعي و كفاءة التحويل الغذائي مقارنة بمجموعة السيطرة. كما اظهرت النتائج تفوق المعاملة الثالثة معنويا ($P \leq 0.05$) في مستوى الاضداد المناعية في مصل دم الافراخ ضد مرض النيوكاسل في الايام (9 ,19, 29 و39) من عمر الافراخ مقارنة بباقي معاملات التجربة التي تفوقت هي الاخرى معنويا في نفس الفترات السابقة على مجموعة السيطرة .

الكلمات المفتاحية: حقن البيض ، فروج اللحم ، فيتامين E ، زيت كبد الحوت، الاستجابة المناعية.