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Original Article

Effect of ND Vaccine, Multivitamins AD_3E, and Omega-3 on Performance and Immune Response of Broilers

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Abstract

This field study intended to compare between in ovo injection of Newcastle disease (ND) killed vaccine, multivitamins AD_3E, and omega-3 with their supplying nutrients by feed and use conventional ND vaccination on performance and immune response of two strains of broilers. Eggs of two commercial broiler strains were used in this experiment (Ross 308 and Cobb 500). On day 18 of incubation, three hundred fertilized eggs from each strain were distributed into three groups (100 eggs for each group). The first group was injected with 0.1 ml saline solution and acted as a control (T1), (T2) was injected with a mixture of (0.1 ml ND vaccine, 0.1ml multivitamins AD_3E and 0.1ml omega-3 oil) and (T3) was injected with 0.1 ml saline solution. After hatching, all hatched chicks were distributed into three equal groups and each group subdivided into two replicates. All chicks in T1 and T2 were fed on a standard diet, while chicks in T3 were feed on basic component diet lack of 0.25% of fat source and supplemented with omega-3 plus AD_3E (50gm/100kg), until the end of the experiment. Traits involved hatchability, body weight, weight gain, feed intake, feed conversion ratio and antibody titer against ND virus. Results revealed that hatchability, body weight, weight gain, and antibody titer against ND virus and feed conversion ratio were improved significantly ($p \leq 0.05$). However, feed intake were reduced significantly in T2 and T3, as compared with a control group of the two strain (Cobb and Ross). Therefore, using in ovo injection of ND vaccine, AD_3E and omega-3 for improving a hatchability, performance, and antibody titer against ND virus are highly recommended.

Keywords:In ovo injection, Broiler, Omega-3, Multivitamins AD_3E, Immune response, ND vaccine

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Introduction

Hatchability, healthy chicks and fast growing may be possible increasing with in ovo technology through early feeding and vaccination of the developing embryo. Exogenous fatty acids and

antioxidants provided during incubation may enhance polyunsaturated fatty acid (PUFAs), and antioxidant status of the chicken embryo (Schaal, 2008 and perez et al., 2010). Fats are included in poultry diets to meet the necessary nutrients and energy needs required for growth (Leeson and Summers, 2005). Polyunsaturated fatty acids considered essential because they cannot be synthesized by body, so it must be obtained from the diet (Woods et al., 2005). Polyunsaturated fatty acids are important constituents of the immunity of the chicks because polyunsaturated fatty acids in the diet will determine the eicosanoids formed (Stulnig, 2003). Eicosanoid activity depends on the ratio and content of omega-6 and omega-3 fatty acids (Calder, 1998). The presence of balanced omega-6: omega-3 fatty acids in poultry diets are essential for normal growth and development and other biological functions (FAO, 2010). High bioavailability of omega-6 fatty acids, leads to a production of pro-inflammatory eicosanoids increasing the incidence of inflammatory related disorders in poultry (Calder, 1998). While, omega-3 fatty acids possess anti-inflammatory or less inflammatory properties by decreasing the release of pro-inflammatory eicosanoids and cytokines (Stulnig, 2003). Cytokines produced by white blood cells serve as regulators to the whole body by exertion of different effects on lymphocytes and other immune cells in response to infection and injury. From the human health aspect, omega-3 fatty acids are essential for playing important role in the prevention of coronary heart disease, hypertension, inflammatory, autoimmune disorders and cancer (El- Yamany et al., 2008). As well as, omega-3 fatty acids were improved immunity, performance, lipid profile besides increasing in market weight (Al-Zuhairy and Alasadi, 2013, Jameel and Sahib, 2014). (Tobarek et al.; 2002, Al-Mayah, 2009) Indicated that titer of antibody against Newcastle disease (Lasota strain) was increased in broilers at the age 35 days fed a ration contained omega-3. Supplementing the hen's diet with oils containing beneficial omega-3 fatty acids and high levels of antioxidants can be costly. The ability to direct supply growing embryos with specific compounds may decrease the need for the long term formulation of enriched rations for maternal diets to achieve a similar effect; therefore, in ovo injections may also provide a more accurate dose at a specific time for peak absorption of specific nutrients by the embryo (Schaal, 2008).

As such, in ovo administration of high quality fatty acids may prove beneficial for improving energy production during embryogenesis and hatching. The use of antioxidants, especially vitamin E (VE) has been proven to reduce harmful peroxidation of lipids and cholesterols in animal models (Singh, et al., 2005). Developed and improved nutritional status afforded by in ovo feeding subsequently improved hatchability, and broiler performance (Al-Murrani, 1982; Ohta et al, 1999; Bakyaraj et al., 2011; Selim et al., 2012; Al-Zuhairy and Alasadi, 2013, Jameel and Sahib, 2014) and immune responses (Konashi et al., 2000; Bhanja and Mandal, 2005; Selim et al., 2012; Al-Zuhairy and Alasadi, 2013) besides increasing body weight at market age. Therefore, the present experiment was designed to investigate the effects of in ovo injection with Newcastle disease vaccine, multivitamins AD_3E, and omega-3 on broiler performance and immune response against ND virus.

Material and Methods

Experimental design

The experiment was conducted on 300 fertilized eggs of two commercial broiler strains (Cobb 500 and Ross 308). All eggs were distributed into three treatments 100 eggs per each treatment. The incubation period was carried out at Al-Saud Hatchery in the holy city of Karbala. All fertilized eggs for each experiment were set in the trays in the same incubator. On day 18 of incubation when the

egg transferred from incubators to hatchers, (Previously, it was approved that day 18 of incubation was the best time of in ovo injection ,Sharma and Burmester, 1982), all eggs were removed from the incubator simultaneously to facilitate in ovo injections. The eggs were first candled to remove non-fertile. The surface of each egg was cleaned with 70% ethanol and a small puncture were made in all eggshells by innovated injected egg machine (Figure 1) (Jameel, 2013). The device works by two system: the first was electrical (to fill the compressor with air) and the second was aeriform which consist from air compressor, dental foot control, dental handpiece tubing, dental high speed handpiece (450000 rpm) which used by the dentist, and dental diamond bure (make a hole in the egg shell).

Figure (1) shows the innovated injected egg machine

The eggs were injected through 23 gauge, 1.25 inch needle and automatic injector used to administer all injections into the amnion of the egg. (100 eggs for each group) were injected with 0.1 ml saline solution as a control (T1), while (T2) were injected with mixture of (0.1 ml ND vaccine , 0.1ml multivitamins AD_3E and 0.1ml omega-3 oil) and (T3) were injected with 0.1 ml saline solution. Upon completion of all injections, all eggs were returned to the hatcher until the day of hatching. Day of hatching, all day- hatched chicks boxed and transported from hatchery to poultry farm of college of veterinary medicine/ Baghdad University and distributed into three treatments . Each treatment group was further sub-divided into 2 replicates (25 birds per replicate). All chicks in T1 and T2 were fed on a standard diet, while chicks in T3 were fed on basic component diet lack of 0.25% of fat source and supplemented with omega-3 plus AD_3E (50gm/100kg), until the end of the experiment.

Feeding program

Feed and water provided in *ad-Libitum* during the experiment. A two-phase feeding program consists of offering a starter (1-21 days of age) and finisher (22-35 days of age) was provided to the broilers. Diets were formulated to meet or exceed requirements by the National Research Council (NRC, 1994) (Table 1). Light was provided the whole day long with only one hour cut off to get them used to the darkness.

Production traits

Production traits measured in this study were hatchability, final body weight (35 days of age), feed intake, body weight gain and feed conversion ratio. The latter three traits were determined weekly and the data presented as a total mean for the whole experimental period (5 weeks).

Blood samples collection and laboratory analysis

On day 9th, 19th, and 29th of age, blood samples were collected from five birds in each replicate from the bronchial vein in a test tube without anticoagulant. The blood allowed to clot and centrifuged for 10 minutes/3000 rpm. Serum was collected and stored in deep freeze (-20) until analysis. Determination the level of antibody in the serum was done using Proflok ELISA Kit (Synbiotics–USA)and according to the manufacturer's instructions.

Statistical analysis

Data generated from experiment was carried out in a complete randomized design (Steel and Torrie, 1980). These data were subjected to ANOVA according to general linear model procedure of SPSS software (SPSS, 2001). The significant differences among means were determined by Duncan's multiple range tests with ($p \leq 0.05$) level of significance.

Table (1) compositions of experimental diet according to (NRC, 1994)

Ingredient %	Starter diet		Finisher diet	
	T1& T2	T3	T1& T2	T3
Yellow corn	36	36	44	44
Soybean meal(48% protein)	30	30	26	26
Wheat	26	26	20	20
Protein concentrate	5	5	5	5
Sunflower oil	1.5	1.25	3.5	3.25
Flaxseed oil"	-	0.25	-	0.25
Multivitamin AD_3E	-	0.05	-	0.05
Premix*	0.1	0.1	0.1	0.1
Lime stone	1	1	1	1
Salt	0.3	0.3	0.3	0.3
Dicalcium phosphate	0.1	0.1	0.1	0.1
Total	100	100	100	100
Calculated chemical analysis				
Metabolize energy (kcal/kg)	2926	2926	3097.8	3097.8
Crude protein (%)	22.4	22.4	20.5	20.5
Calcium (%)	0.82	0.82	0.80	0.80
Available phosphorus (%)	0.61	0.61	0.58	0.58
Methionine (%)	0.61	0.61	0.58	0.58
Lysine (%)	1.74	1.74	1.63	1.63

* Premix produced in Jordan (VAPCO®) which contains: vit A 8000000 IU; vit D3 1500000 IU; vit E 1000 IU; vit K3 2000 mg; vit B1 500 mg; vit B2 500 mg; vit B6 200 mg; vit B12 8 mg; ca pantothenate 400 mg; nicotinamide 6000 mg; folic acid 50 mg; methionine 13 mg; lysine 61 mg; aspartic acid 92 mg; glutamic acid 166 mg; cysteine 1 mg; valine 40 mg; tyrosine 9 mg; glycine 382 mg; arginine 117 mg; leucine 48 mg; phenylalanine 40 mg; Mn sulphate 0.40 gm; zinc sulphate 0.15 gm; iron sulphate 0.50 gm; copper sulphate 0.04 gm; cobalt chloride 0.01 gm.

Results and discussion

The effects of different treatments on hatchability and weight of hatching chicks for two strains (Ross 308 and Cobb 500) are shown in (Table 2 and 3). The hatchability and weight of hatching chicks were significantly ($p \leq 0.05$) increased in T2, which was (88% in Cobb strain) and

(89% in Ross strain) in T3 as compared with the control group (82%, 84%) for Cobb and Ross strains respectively. The broiler body weight and body weight gain were significantly ($p \leq 0.05$) increased and feed conversion ratio was improved in T2 and T3 for Cobb and Ross strains respectively as compared with the control group. While, feed consumption were significantly ($p \leq 0.05$) decreased in T2 and T3 respectively as compared with the control group for Cobb and Ross strains (table 2 and 3). The effects of different treatments on antibody titer against Newcastle disease virus were presented in table (4 and 5). ELISA antibody titer against ND virus were significantly ($p \leq 0.05$) increased in T2 and T3 respectively as compared with the control for Cobb and Ross strains at day 9, 19, and 29 old chicks.

Table (2) Effect of different treatments on hatchability, weight of hatching chicks, body weights, weight gain, feed intake, and feed conversion ratio of Cobb strain. Mean ± SE

Parameters \ Treatments	T1	T2	T3
Hatchability %	82	88	82
Weight of hatching chicks (gm)	40.25±0.37 A	40.87±0.26 B	40.25±0.50 A
Body weight (gm)	1861.12±2.7 C	2103.62±3.4 A	2004.25± 3.23 B
Weight gain (gm)	1820.62±2.63 C	2062.87±2.3 A	1963.87±2.14 B
Feed intake (gm)	3145.0±2.99 A	3076.50±2.61 C	3115.50±2.50 B
Feed conversion ratio	1.72 ± 0.004 A	1.49 ± 0.001 C	1.58 ± 0.003 B

Different letters in the same raw denoted significant differences between treatments at a level ($p \leq 0.05$).

Table (3) Effect of different treatments on hatchability, weight of hatching chicks, body weights, weight gain, feed intake, and feed conversion ratio of Ross strain. Mean ± SE

Parameters \ Treatments	T1	T2	T3
Hatchability %	84% B	89% A	84% B
Weight of hatching chicks (gm)	40.29±0.61 A	40.70±0.72 B	40.41±0.49 A
Body weight (gm)	1859.37±3.64 C	2099.87±3.09 A	1988.75±2.10 B
Weight gain (gm)	1819.12±3.65 C	2059.0±3.13 A	1948.50±2.98 B
Feed intake (gm)	3121.50±3.19 A	3052.50±3.50 B	2993.50±1.65 C
Feed conversion ratio	1.71 ± 0.005 A	1.48 ± 0.003 C	1.53 ± 0.007 B

Different letters in the same raw denoted significant differences between treatments at a level ($p \leq 0.05$).

Table (4) Effect of different treatments on antibody titer against ND virus at 9, 19, and 29 day old chicks of Cobb strain. Mean± SE

Hatchability were Increased with in ovo technology could be due to the action of high quality fatty acids, which might improved the production of energy during embryogenesis. The in ovo feeding of VE (exogenous vitamin E) around the last quarter of incubation could be beneficial in reducing the production of free radicals that cause damage of cellular membranes. The results of this study is in

agreement with the suggestion of (Surai, 2000; Puthpongsiriporn et al., 2001; Singh, et al., 2005; Schaal, 2008). Previous study, were recorded that the use of antioxidant especially VE reduce harmful peroxidation of lipids and cholesterols in animal models. Another studies, (Bakyaraj et al., 2011 and selim et al; 2012; Al-Zuhairy and Alasadi, 2013) have also shown that nutrient administration "in ovo" could be considered as an alternative method to improve hatchability.

Treatments / Parameters	T1	T2	T3
9 day	2523.4±52.17 C	3332±18.86 A	2607.2±7.45 B
19 day	2649.4±17 C	3610.4±9.94 A	2852.8±16.27 B
29 day	1804.8±5.63 C	2609±10 A	2025.4±9.70 B

Different letters in the same raw denoted significant differences between treatments at a level ($p \leq 0.05$).

Table (5) effect of different treatments on antibody titer against ND virus at 9, 19, and 29 day old chicks of Ross strain. Mean± SE

Treatments / Parameters	T1	T2	T3
9 day	2749.4±52.16 B	3602±18.86 A	2767.3±7.45 B
19 day	2599.4±17 C	3770.4±9.94 A	2963.8±16.27 B
29 day	1754.8±5.63 C	3391±10 A	2904.4±9.70 B

Different letters in the same raw denoted that significant differences between treatments at a level ($p \leq 0.05$).

In this study the mean body weight, and body weight gain were increased significantly. In addition feed conversion ratio was improved significantly. However, the significant reduction in feed consumption could be due to in ovo feeding omega-3 and AD_3E vitamins which might improve the energy availability for the developing embryo and protecting the cellular membranes. The fatty acid reserves from free radicals and peroxidation might be lead to improve the embryo's ability to hatch and to perform; therefore, supplying embryos with exogenous nutrients in ovo could be increased final body weight of broilers through modulating gut morphology of the embryo. Also fats rich with omega-3 increased growth due to activate of bile which lead to increase digestion of fats in the intestine, and increase efficiency of digestion and absorption of diets in the intestine and lead to more useful from the diet. This results is in compatible with the suggestion of (El-Sayed and Hashim, 2000; Uni and Ferket, 2003; Al-Zuhairy and Alasadi, 2013).

The increase of antibody titer against ND vaccine could be due to their combination between ND vaccine and immunomodulator multivitamins AD_3E and omega-3. These components are important for the development of the immune cell structure and eicosanoid formation. In addition, omega-3 PUFAs have anti-inflammatory by decreasing the release of pro-inflammatory eicosanoids and cytokines. Therefore, supplying of omega-3 PUFAs during in ovo may impact the development of a strong immune system in birds and increase antibody production. Korver and Klasing, 1997 found that increasing dietary omega-3 (LNA) inhibited

the conversion of omega-6 (LA) to long chain omega-6 FA in immune tissues. Also, competition between LA and LNA in conversion to long-chain FA and eicosanoids in immune tissues most likely contributed to improve antibody production in response to vaccines (Wang *et al.*, 2002 and Puthpongsiriporn and Scheideler, 2005). Furthermore, (Wang *et al.*, 2004) reported that, LA to LNA ratio may influence in the binding activity of IgG-receptor on the yolk sac membrane and thus it affects the maternal-embryo transfer of yolk IgG. The increment total IgG and specific antibody IgG in the embryo circulating system during the incubation with the increment LNA or decrement LA to LNA ratio could benefit young chicks by improving the capability of immune defense (Radwan *et al*., 2012). This result is in agreement with the suggestion of (Bhanja *et al*; 2006 and Bakyaraj *et al*; 2011; Al-Zuhairy and Alasadi, 2013), who evaluated the early post-hatch growth and immunity through in ovo supplementation of nutrients like fatty acids and vitamins AD_3E.

In conclusion, in ovo injection of combination ND vaccine, multivitamins AD_3E, and omega-3 oil revealed best beneficial effect on hatchability, productivity, and immunological traits besides increasing body weight at marketing age.

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