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Effect of ND Vaccine, Multivitamins AD$_3$E, 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 AD3E, 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 (0.1ml multivitamins AD3E and 0.1ml omega-3 oil), 0.1 ml ND vaccine 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 AD3E (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 was reduced significantly in T2 and T3, as compared with a control group of the two strains (Cobb and Ross). Therefore, using in ovo injection of ND vaccine, AD3E and omega-3 for improving a hatchability, performance, and antibody titer against ND virus are highly recommended.

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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 (0.1ml multivitamins AD_3E, and 0.1ml omega-3 oil), 0.1 ml ND vaccine 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) and 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 AD3E (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 9^{th}, 19^{th}, and 29^{th} 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.

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 (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.

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

Treatments / Parameters	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 ($p \leq 0.05$).

Hatchability were Increased with in ovo technology could be due to the action of high quality fatty acids, which might improve 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 study, (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, which lead to 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. These results is in compatible with the suggestion of (El-Sayed and Hashim, 2000; Uni and Ferket, 2003; Al-Zuhairy and Alasadi, 2013).

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

Treatments / Parameter	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

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 ($p \leq 0.05$).

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 AD3E vitamins.

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).

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 / Parameter	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 ($p \leq 0.05$). 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; 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 AD3E. 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.

References

AL-Mayah AA. (2009). Effect of fish oil on humoral immunity of broiler chicks. Bas. J. Vet. Res.8(2)**.**

Al-Murrani WK. (1982). Effect of injecting amino acids into the egg on embryonic and subsequent growth in the domestic fowl. Br. Poult. Sci. 23: 171-174.

Al-zuhairy MA. and Alasadi Yj. (2013). Effect of in ovo injection with Newcastle disease vaccine, multivitamins AD3E, and omega-3 on performance and immune response of broiler. *International Journal of advanced Biological Research*. 3(2): 208-211.

Bakyaraj S, Subrat KB, Samir M and Banabihari D. (2011). Modulation of post-hatch growth and immunity through in ovo supplemented nutrients in broiler chickens. *J Sci Food Agric* 2012. 92: 313–320.

Bhanja SK, Mandal AB, Agarwal SK and Majumdar S. (2006). Modulation of post hatch growth and immune competence through in ovo injection of vitamin E and linoleic acid. (Supplement, European Poultry Conference. World's Poult Sci J. 62:325.

Bhanja SK and Mandal AB. (2005). Effect of in ovo injection of critical amino acids on pre and post-hatch growth, immunocompetence and development of digestive organs in broiler chickens. Asian. Aust. J. Anim. Sci., 18: 524-531.

Calder PC. (1998). Immunoregulatory and anti-inflammatory effects of n-3 polyunsaturated fatty acids. *Brazilian Journal of Medical and Biological Research*.31: 467-490.

El-Sayed EM and Hashim ME. (2000). Effect of Nigella sativa on the immune response to Eimeria vaccination in chicken. Egypt. J. Agri. Res. 78 (1): 231-239.

El-Yamany A. T, El-Allawy HM, El-Samee LD and EL-Ghamry AA. (2008). Evaluation of using different levels and sources of oil in growing Japanese quail diets. *American-Eurasian Journal of Agricultural & Environmental Sciences*. 3(4): 577-582.

Food and Agriculture Organization (F.A.O.). (2010). Fats and fatty acids in human nutrition. Food and nutrition paper. 91: 21-36.

Jameel YJ, and Sahib AM. 2014. Effect of In ovo injection with Newcastle Disease Vaccine, Multivitamins AD_3E, and Omega-3 on Carcass Characteristics of Broilers. MRVSA journal. 3(1): 21-29.

Konashi S, Takahashi K and Akiba Y. (2000). Effects of dietary essential amino acid deficiencies on immunologic variables in broiler chickens. Br. J. Nutri. 83: 449-456.

Korever DR and Klasing KC. (1997). Dietary fish oil alters specific and inflammatory immune responses in chicks. *Journal of Nutrition,* 127: 2039-2046.

Leeson S and Summers JD. (2005). Commercial poultry nutrition.3rd ed. Nottingham university press. England. 229- 296.

National Research Council (NRC). 1994. Nutrient requirements of poultry. 9th ed. National Academy Press. Washington. D. C. USA.

Ohta YN, Tisushima K, Koide K, Kidd MT and Ishibashi T. (1999). Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. Poult. Sci. 78: 1493-1498.

Perez TI, Zuidhof MJ, Renema RA, Curtis JM, Ren Y, Betti M. (2010). Effects of vitamin E and organic selenium on oxidative stability of ω-3 enriched dark chicken meat during cooking. Journal of Food Science. 75: 25–34.

Puthpongsiriporn U and Scheideler SE. (2005). Effects of dietary ratio of linoleic to linolenic acid on performance, antibody production and in vitro lymphocyte proliferation in two strains of Leghorn pullet chicks. Poultry Sci. 84: 846-857.

Puthpongsiriporn V, Scheideler S, Sell JL and Beck MM. (2001). Effect of vitamin E and C supplementation on performance, In vitro lymphocyte proliferation, and antioxidant status of laying hens during heat stress. Poult Sci. 80:1190-1200.

Radwan NL, Abd El-Samad MH and Sherin A. (2012). Effects of different dietary rations of linoleic acid to alinolenic acid on productive performance, immunity of laying hens and egg yolk fatty acid composition. *Egypt Poultry Sciences*. 32: 163-188.

Schaal TP. (2008). The Effects of In ovo Feeding of Fatty Acids and Antioxidants on Broiler Chicken Hatchability and Chick Tissue Lipids. University Honors College, Oregon State University**.**

Selim Sh A, Gaafar KM and El-ballal S S. (2012). Influence of in ovo administration with vitamin E and ascorbic acid on the performance of Muscovy ducks. Emir. J. Food Agric. 24 (3): 264-271.

Sharma JM and Burmester BR. (1982). Resistance to Marek's disease at hatching in chickens vaccinated as embryos with the turkey herpesvirus". Avian Dis. 26:134-149.

Singh U, Devaraj S and Jialal I. (2005). Vitamin E, oxidative stress, and inflammation. Annu. Rev. Nutr. 25:151–174**.**

Statistical Packages for the Social Sciences (SPSS). (2001). Statistical software for windows version 11.Microsoft. Chicago. I. L. USA.

Steel RG and Torrie JH. (1980). Principle and procedures of statistics.2nd ed. McGraw-Hill Book Co. Inc. New York. USA. 183-193.

Stulnig TM. (2003). Immunomodulation by polyunsaturated fatty acids: Mechanisms and effects. *International Archives of Allergy and Immunology*. 132: 310-321.

Surai PF. (2000). Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. Br. Poult. Sci. 41:235-243.

Tobarek M, Lee YW, Garrido R, Kaiser S and Henning B. (2002). Unsaturated fatty acids selectively induce an inflamm- atory environment in human endothelial cells.Amer. J. Clin. Nut. 75: 119-125.

Uni Z, Ferket PR. (2003). Enhancement of development of oviparous species by in *ovo* feeding. North Carolina State Univ. and Yissum Research Development Company Assignees. US Pat. 6:592,878.

Wang YW, Sunwoo H, Cherian G and Sim JS. (2004). Maternal dietary ratio of linoleic acid to α- linolenic acid affects the passive immunity of hatching chicks. Poultry. Sci. 83: 2039-2043.

Wang Y, Ajugah AO, Sunwoo HH, Cherian G and Sim JS. (2002). Maternal dietary n-3 fatty acids alter the spleen fatty acid composition and bovine serum albumin induced wing web swelling in broilers. Poult. Sci. 81: 1722-1727.

Woods VB, Forbes EGA, Easson DL and Fearon AM. (2005). Dietary source of unsaturated fatty acids for animals and their subsequent availability in milk, meat and eggs. Occasional Publication No. 4.Agri-Food and Biosciences Institute. Global Research Unit. Belfast. Northern Ireland.