INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

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EFFECT OF IN *OVO* INJECTION WITH NEWCASTLE DISEASE VACCINE, MULTIVITAMINS AD_3E, AND OMEGA-3 ON PERFORMANCE AND IMMUNE RESPONSE OF BROILER

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ABSTRACT
A study was carried out to investigate the influence of in *ovo* injection Newcastle disease killed vaccine, exogenous omega-3 (flaxseed oil), and multivitamins AD_3 E into an egg embryo on performance and immune response of broiler. On day 18 of incubation when the eggs transferred from incubator to hatcher, 400 fertilized eggs (100 eggs for each group) were injected with 0.1 ml saline solution as a control (T1), 0.1 ml ND vaccine (T2), 0.1 ml ND vaccine plus 0.1 multivitamins AD_3E (T3), and 0.1 ml ND vaccine plus 0.1ml omega-3 (T4). After hatching, all hatched chicks boxed and transported from hatchery to a poultry farm. Chicks were distributed into four groups and each group subdivided into two replicate. Traits involved in this study were hatchability, body weight, weight gain, feed intake, feed conversion ratio, and antibody titer against ND virus of 9, 19, and 29 day old chicks. Results revealed that groups within *ovo* injection of ND vaccine plus omega-3 (T4), and in *ovo* injection of ND vaccine plus multivitamins AD_3E (T3) have a significant ($P < 0.05$) increasing in hatchability, body weight, weight gain, and antibody titer against ND virus also feed conversion ratio was improved significantly ($P < 0.05$).

KEYWORDS: in *ovo* injection, egg, broiler, early nutrition, early vaccination, omega-3, multivitamins AD_3E.

INTRODUCTION

Hatchability in broiler eggs has not increased over the last twenty years (Schaal, 2008). Genetic selection, nutrition, and management of poultry flocks has improved over the last twenty years, hatchability of broiler eggs set in commercial hatcheries has not increased (Schaal and Cherian, 2007). Increasing hatchability, healthy and fast growing with in *ovo* technology may be possible through feeding metabolic modulators to the developing embryo. Exogenous fatty acids and antioxidants provided during incubation may enhance polyunsaturated fatty acid (PUFAs), lipid, and antioxidant status of the chicken embryo (Schaal, 2008 and perez *et al*., 2010). The use of antioxidants, especially vitamin E has been proven to reduce harmful peroxidation of lipids and cholesterols in animal models (Singh, *et al*., 2005), therefore, developed and improved nutritional status afforded by *in ovo* feeding subsequently improved hatchability percentage, hatching weight, and growth performance (Bakyaraj *et al*., 2011; Selim *et al*., 2012) and immune responses (Al-Rubee, 2011; Selim *et al*., 2012) besides increasing the market weight.

MATERIALS & METHODS

An experiment was conducted on 400 fertilized commercial broiler eggs (Cobb strain). All eggs were distributed into four treatments 100 eggs per each treatment. The incubation period was carried out at Al-Saud Hatchery inthe 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; all eggs were removed from the incubator simultaneously to facilitate the in *ovo* injections. Non-fertile eggs were removed, whereas the fertilize eggs were cleaned with 70% ethanol then eggshells were punched by machine of my design which can make a hole in the egg shell to conduct the injection. 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 fertilized eggs in *ovo* injected with 0.1ml saline solution as control group (T1), 100 fertilized eggs in *ovo* injected with 0.1ml killed Newcastle disease vaccine, 100 fertilized eggs in *ovo* injected with 0.1ml killed Newcastle disease vaccine and 0.1 ml multivitamin AD3E, and 100 fertilized eggs in *ovo* injected with 0.1ml killed Newcastle disease vaccine and 0.1 ml omega-3. Upon completion of all injections, all eggs were returned to the hatcher until the day of hatching. After hatching, all hatched chicks boxed and transported from hatchery to poultry farm in College of Veterinary Medicine / University of Baghdad and distributed into treatment and each treatment group was further sub-divided into 2 replicates.

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 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).

On day 35 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 at 3000 rpm to obtain on serum which stored in deep freeze (-20) until analysis. Serum was performed according to the manufacturer's instructions listed in the Proflok ELISA Kit (Synbiotics–USA), which is a rapid serological test for the detection of antibody in chicken serum samples. All Data were analyzed statistically by using analysis of variance (ANOVA) and means compared for significance using least significant difference (L.S.D) for comparison of means on a computer program by using SPSS Program (Snedecor and Coehran, 1980).

TABLE 1: compositions of experimental diet (starter and finisher) according to (NRC, 1994)

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

The effects of in *ovo* injection with ND vaccine, multivitamin AD_3E, and omega-3 on hatchability were presented in table (2) and increased significantly ($p \leq 0.05$) in T4 (in *ovo* injected with ND vaccine and omega-3) and T3 (in *ovo* injected with ND vaccine and AD_3E) respectively as compared with T2 (in *ovo* injected with ND) and with a control group.

TABLE 2: effect of in *ovo* injection on hatchability, body weights, weight gain, feed intake, and feed conversion ratio (gm). Mean ± SE

Treatments	T1	T2	T3	T4
Parameters	Injected with saline	Injected with ND vaccine	Injected with ND + multi Vit. AD_3E	Injected with ND + Omega-3
Hatchability %	82%	81%	84%	87%
	C	C	B	A
Body weight (gm)	1861.12±2.13	1852.0±2.25	1983.87±2.15	2062.5±3.62
	C	D	B	A
Weight gain (gm)	1820.62±1.64	1811.75±2.34	1943.37±2.51	2021.75±3.16
	C	D	B	A
Feed intake (gm)	3145.0±2.68	3136.50±3.50	3087.0±2.5	3096.50±1.56
	A	B	D	C
Feed conversion ratio	1.72 ± 0.004	1.73 ± 0.002	1.59 ±0.004	1.53 ± 0.003
	A	A	B	C

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

Final body weight, and weight gain, were presented in table (2) and increased significantly ($p \leq 0.05$) in T4 (in *ovo* injected with ND vaccine and omega-3) and T3 (in *ovo* injected with ND vaccine and AD_3E) also T2 (in *ovo* injected with ND) respectively as compared with the control group. However, feed intake and feed conversion ratio were reduced significantly ($p \leq 0.05$) in T4, and T3, respectively as compared with T2 and control group. The effects of in *ovo* injection with ND vaccine, multivitamin AD_3E, and omega-3 on antibody titer against Newcastle

disease virus were presented in table (3) and increased significantly ($p \leq 0.05$) in T4 (in *ovo* injected with ND vaccine and omega-3) then T3 (in *ovo* injected with ND vaccine and AD_3E) and T2 (in *ovo* injected with ND) respectively as compared with control group at day 9, 19, and 29 old chicks.

TABLE 3: effect of in *ovo* injection on antibody titer against ND virus at 9, 19, and 29 day old chicks. Mean± SE

Treatments	T1	T2	T3	T4
Age	Injected with saline	Injected with ND vaccine	Injected with ND + multi vit AD_3E	Injected with ND + Omega-3
9 day	2523.4±52.16	2605.8±9.34	2711.6±5.89	3134.4±18.75
	D	C	B	A
19 day	2649.4±17.04	2750.6±14.59	2825.8±7.89	3250.6±23.89
	D	C	B	A
29 day	1804.8±5.63	2105.8±7.23	2219.8±21.08	2253.4±14.49
	C	B	AB	A

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

Hatchability were Increased with in *ovo* technology could be due to in *ovo* administration of high quality fatty acids may beneficial for improving energy production during embryogenesis and hatching also in *ovo* feeding of VE (exogenous vitamin E) administered around third part of incubation could be beneficial in reducing the production of free radicals that cause a serious damage to the cellular membranes, therefore, in *ovo* injections may also provide a more accurate dose at the specific time for peak absorption of specific nutrients by the embryo. The result obtained in the experiment was in agreement with the suggestion of (Surai, 2000; Puthpongsiriporn *et al*., 2001; Singh, *et al*., 2005; Schaal, 2008) they were recorded that the use of antioxidant especially VE reduce harmful peroxidation of lipids and cholesterols in animal models. In a recent study, (Bakyaraj *et al*., 2011 and selim *et al*., 2012) have also shown that nutrient administration in *ovo* could be considered as an alternative method to improve hatchability. Mean body weight, and body weight gain were increased significantly and feed conversion ratio was improved significantly. However, feed consumption was reduced significantly could be due to in *ovo* feeding omega-3 and AD3E vitamins may improve the energy availability for the developing embryo and protecting the cellular membranes and fatty acid reserves from peroxidation might be lead to improve the embryo's ability to hatch and to perform to its genetic potential; therefore, Supplying embryos with exogenous nutrients in *ovo* could be increased final body weight of broilers through modulating embryo gut morphology. 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 intestine lead to more useful from the diet. The results obtained were in agreement with the suggestion of (El-Sayed and Hashim, 2000; Uni and Ferket, 2003). The increase of antibody titer against ND vaccine could be due to their combination between ND vaccine and immunomodulator multivitamins AD_3E or/and omega-3 because fatty acids are important constituents of the immune cell structure and eicosanoid formation. Also omega-3 PUFAs possess anti-inflammatory or less inflammatory properties by decreasing the release of pro-inflammatory eicosanoids and cytokines, Therefore, dietary supply of omega-3 PUFAs during the early post hatch may impact the development of a strong immune system in birds may increase antibody production. The result obtained were in agreement with the suggestion of (Bhanja *et al*., 2006 and Bakyaraj *et al*., 2011) who reported that early post-hatch growth and immunity were assessed through *in ovo* supplementation of nutrients like fatty acids and vitamins AD3E.

CONCLUSION

It can be concluded from this study that in *ovo* injection of ND vaccine with omega-3 or with multivitamins AD_3E could improve hatchability, and productive performance of broilers.

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