Effect of injection hatching eggs with Newcastle disease vaccine and different doses of vitamin E on some productive traits and immune response of broilers

Mushtaq T. Abdulwahid
Department of Veterinary Public Health, College of Veterinary Medicine, Baghdad University, Iraq.
E-mail: dr.m.t.abdulwahid@gmail.com
Accepted: 15/10/2015

Summary

This study aimed to investigate the effects of injection hatching eggs with different doses of vitamin E on productive traits, some physiological and immune response to Newcastle disease vaccine. Two hundred fertile eggs of broiler breeder Ross (308) strain were incubated in automatic incubator machine after divided into four treatments (50 eggs per treatment) with two replicates. First treatment was injected 0.1ml/egg of phosphate buffer sterile into amniotic fluid at day 18th of incubation which it was considered as control group, second treatment was injected with 0.1ml/egg of inactivated Newcastle disease vaccine, third treatment was injected with 0.1ml/egg of inactivated Newcastle disease vaccine and 0.1 ml/egg of vitamin E, fourth treatment was injected with 0.1ml/egg of inactivated Newcastle disease vaccine and 0.15 ml/egg of vitamin E. All injected eggs were carried back into incubator for complete hatching process. Hatched chicks were transferred to the hall in farm of the Veterinary Medicine College/University of Baghdad; therefore, the chicks were distributed into four treatments with two replicates depending on the previously treated groups until fifth week of age. The results revealed that the treated groups with vitamin E were significantly ($P \leq 0.05$) increased in hatchability percentage, body weight, weight gain, as well as significant reduce in feed intake and improvement in feed conversion ratio as compared with second group and control, the results of third group showed significantly increased ($P \leq 0.05$) in antibody titers against Newcastle disease virus at aged (21 and 35) day as compared with the other treated groups and control.

Keywords: Injection hatching eggs, Vitamin E, Immune response, Broiler chickens.

Introduction

The rapid growing of modern breeds makes suffering less supply of energy, less supply of some vitamins and minerals which led to decrease in hatchability and vitality of newly hatched chicks (1). Many researchers were administrated that possible increasing hatchability, vitality with enhance growing with early nutrition of chicken embryos through exogenous antioxidant and nutrient solution by in ovo technology (2 and 3) where that procedure improved feeding metabolic modulators to developing embryos (4) however, supplement of vitamins have big role in embryonic development (5). Vitamin E works to maintain the integrity of body tissues and preserve of cells membranes from damages as a good natural antioxidant (6). The efficiency of feeding breeders has a negative impact on the productive performance of the hatched chicks and reduces resistance to disease (7). Vitamin E has a role in stimulated the immune system as an active natural antioxidant in the body, It works to prevent the body cells damages by prevent the oxidation of unsaturated fatty acids to maintain the membranes of immune cells which was led to stimulate cellular and humoral immunity and gave body the ability to resist different bacterial infection (8 and 9). Vitamin E has a positive role on the productive performance as high rate of body weight and reduces feed consumption rate and improvement in the feed efficiency (10). In the other study the results of treated group with inject 50 µl vitamin E in embryos at 18 days of age showed that highest significantly ($P \leq 0.05$) in the productive performance of the hatched chicks represented by increasing body weight, weekly weight gain and significant reduce in feed intake and improvement in feed conversion ratio as compared with control, also, the same treated group recorded a significant ($P \leq 0.05$) increase in antibody titers against Newcastle disease (ND) vaccine compared with the other treatments and the control group (11). The aim of this study is to investigate the influence of injection hatching eggs of broiler breeders

with different doses of vitamin E on hatchability, some physiological, productive traits and immune response to Newcastle disease vaccine.

Materials and methods

The experimental design of this study includes two stages; the first stage started on 27/3/2014 with entrance of 200 fertile eggs of Ross 308 broiler breeders were obtained from poultry breeding farm of Public Company for Agricultural researches. Then, the eggs were brought in good condition to Department of Biology and Medicine Supervision/Veterinary Directorate. All the eggs were incubated in automated incubator after graded and culled the abnormal and misshapen. The chosen eggs were distributed into four treatments; fifty fertilized eggs were assigned to each treatment with two replicates per treatment. First treatment was injected 0.1ml/egg of phosphate buffer sterile PBS into amniotic fluid at day 18th of incubation which it was considered as control group, second treatment was injected with 0.1ml/egg of inactivated ND vaccine, third treatment was injected with 0.1ml/egg of inactivated ND vaccine and 0.1 ml/egg of vitamin E (oily form) and finally fourth treatment was injected with 0.1ml/egg of inactivated ND vaccine and 0.15 ml/egg of vitamin E. All injected eggs were carried back into incubator for complete hatching process. Hatched chicks were transferred to the hall in farm of the Veterinary Medicine College/University of Baghdad; therefore, the chicks were distributed into four treatments with two replicates depending on the previously treated groups until fifth week of age.

The materials and the vaccine used in this study: Vitamin E (dl-alpha tocopheryl acetate - oily form) was used in ovo injection manufactured by Natural Wealth Nutrition Corporation – USA; Volvac® ND KV (Boehringer Ingelheim- HQ Germany) inactivated oil emulsified vaccine elaborated with Newcastle disease virus, LaSota strain. Each 0.5 ml contains a minimum titer: $10^{8.2}$ EID_{50}/dose of ND before inactivation and Volvac® ND + IB MLV (Boehringer Ingelheim-HQ Germany) is a freeze dried modified live virus vaccine, prepared with LaSota strain of ND and Massachusetts serotype of IB. Titer: not less than $10^{5.5}$ EID_{50} /dose of ND and $10^{3.9}$ EID_{50} /dose of IB.

The fertilized eggs were injected at 18 days of incubation after embryonic inspection by candling with vitality checking and determined the air space for injection. In ovo injection was done by using disposable syringe with needles of length of 2.5 cm (1¼ in), gauge needle 23, the entire length of the needle was extended into the egg to ensure that the needle punctured the amnion (12). The injection was carried out under sterile condition. Immediately after the injection, the site was sealed with sterile paraffin wax and eggs were returned to the incubator (13).

The second stage of this study was started by transporting the newly hatching chicks to the farm of the Veterinary Medicine Collage/University of Baghdad at 16/4/2014. All the hatched chicks were distributed into four groups according to previous treatments with two replicates of each treated group up to 20/5/2014. All chicks were placed in a temperature controlled at 31°C in the first three days, and it was decreased gradually to 23 °C thereafter. Chicks were maintained on 23 hours of light/ day and 1 hours of dark. All birds in the control group and other treated groups were offered feed and water *ad libitum* from placement until the end of this study. The basal diet was formulated as starter diet for 0 to 15 days (Crude protein 23%; metabolic energy 3087 Kcal/Kg) and the grower diet (Crude protein 21%; metabolic energy 3178 Kcal/Kg) up to end of the study. Yellow corn and wheat were the major sources of energy, while the soybean and animal protein were the major sources of protein in these diet nutritional requirements were adjusted according to (14). All groups were vaccinated with 0.03 ml/ chick of attenuated ND+IB (Volvac®) at 7 days old via ocular route.

Blood samples were collected randomly (5 chicks from each replicate) for the physiological, biochemical and immunological tests. Blood samples were withdrawn from heart directly at 7 day of age, after that, blood samples were withdrawn from wing vein by disposable syringe at (21 and 35) days of age, then, added in two glass test tubes, the first with anticoagulant which was potassium

ethylene diamin tetra acetic acid (K_3-EDTA) to measure white blood cells count and hetrophil/ lymphocyte ratio (H/L ratio). After that put it in a refrigerated centrifuge at (2300 rpm) for 5 minutes as recommended (15) to separate serum from the cellular part of blood, that serum was frozen at (-20ºC) until assayed. After calculate hatchability, birds were weighed at one day old by sensitive balance, then weekly by digital computing scale balance until 5th week of age. Chicks growth performance were studied weekly which represented by mean body weight, weight gain, feed intake and feed conversion ratio after hatching up to the end of this study. Biochemical tests were measurement according to (16) by using Kits (Protein total concentration, Aspartate amino transferase (AST) concentration and Alanine amino transferase (ALT) concentration) were produced by BioSystems S.A. company/ Spain, while Albumin kit was produced by Linear Chemicals S.L. Company/ Spain. Antibody titers against ND virus in chicks serum samples were detected at (7, 21 and 35) days of age by using Enzyme Linked Immunosorbent Assay (ELISA) for different groups. ND virus antibody test kit ProFLOK® PLUS Synbiotics Corporation –San Diego/USA was used in this study. All the data obtained were analyzed statistically by using analysis of variance (ANOVA) and least significant differences (L.S.D) were used to be differentiated among mean of results, then the data were calculated as per the statistical program (SPSS) (17).

Results and Discussion

The results showed significant (P≤0.05) increased in hatchability of the third and fourth group as compared with the second group and control, there were the mean (93.3±1.732, 93.0±1.527) respectively as compared with the mean (83.3±0.577, 83.3±1.154) respectively, while, there were no significant (P>0.05) differences between the third and fourth treatments (Table, 1). The significantly increasing in hatchability of the third and fourth groups might be due to in ovo injection with vitamin E which enhancement of the antioxidant status of the embryonic tissues and protect the protein and lipid from oxidative damages. In ovo administration method to 18 day old embryos do not adversely affect hatchability or survival of hatched chicks (18) positively of vitamin E in the late term embryos and it helps the chicks to overcome the hatchling stress. The energy supported the late-term broiler embryos and improves the genetic potential for late embryonic and early post hatch growth. These results were agreement with (9 and 19-22) whom reported that in ovo feeding of various nutrients were improved the energy status of late term embryos and were improved hatchability and early post hatch growth.

Data of (Table, 1) indicated that significantly (P≤0.05) increased in mean body weight and weight gain of third and fourth groups which embryonic injection with vitamin E as compared with the second and control group in the weeks (3rd, 4th and 5th) of the first trait and in the fifth week of the second trait. The third treatment recorded that highly significant (P≤0.05) increased of their traits in the fifth week as compared to the other groups. These findings suggest that vitamin E play as an antioxidant property via to prevent of continues cellular oxidative stress that get inside of the body (23). Vitamin E and Glutathion Peroxidase enzyme are two molecules that help to prevent the oxidative damage (24). Vitamin E prevents the dangerous molecules (peroxides) from being formed, but even with adequate vitamin E, some peroxides evade destruction. Glutathion Peroxidase enzyme destroys the peroxides before they have a chance to cause membrane damage. Glutathion Peroxidase enzyme concentration and activity is directly related to the selenium status of the animal (25). So, vitamin E and Glutathion Peroxidase enzyme preservation body tissues from oxidation damages, Pituitary gland one of these tissues that work to release growth hormone and keep stimulate the body to synthesis the proteins stimulate the body to synthesis the proteins. These results are agreed with (26-29) whom reported that in ovo feeding with nutrients solution might be affected muscles growth through their effect on cell proliferation because they increased protein synthesis and decreased protein degradation. The third and fourth groups recorded a significant (P≤0.05)

decrease in feed consumption in the weeks (2nd and 5th) as compared with the second and control group, also the same (third and fourth) treatments recorded significant (P≤0.05) higher values in feed conversion efficiency as compared with the second and control group up to the end of this in study except the fourth week whereas that no significant differences among groups (Table, 1). The improvement in feed conversion efficiency resulted from the low feed intake of the embryonic injection with vitamin E (third and fourth) groups as compared with the second and control group. These results might be due to the role of vitamin (E) in preserve gastrointestinal tract tissues that led to increase intestinal capacity for good absorption, also the same results were shown increased bird ability to utilize feed ingredients which was reflected by increasing in body weight of those treated groups in spite of limiting in feed consumption. These results agreed with (30 and 31) whom reported that the importance of vitamin E in improving the digestive ability and utilization of necessary elements. While (32) concluded that supplementation of vitamin E to the maternal diets enhancement of the antioxidant status of hatching chicks that maternal dietary enriched with vitamin E would be beneficial in protecting the tissues of the progeny from oxidative deterioration.

Table, 1: Effect of vitamin E in hatchability, body weight, body weight gain, feed intake and feed conversion ratio for different groups. (Mean ± Standard Error).

Groups / Variable	Age	Group 1 (Control) Inject with 0.1ml sterile PBS	Group 2 Inject with 0.1ml inactivated ND vaccine	Group 3 Inject with 0.1ml inactivated ND vaccine and 0.1ml vitamin E	Group 4 Inject with 0.1ml inactivated ND vaccine and 0.15 ml vitamin E
Hatchability%		83.3± 1.154 B	83.3± 0.577 B	93.3± 1.732 A	93.0± 1.527 A
Body weight (gm)	1st day	44.20±0.63	45.62±0.18	47.04±0.36	46.90±0.17
	1st wk	105.2±0.86	110.0±0.70	111.4±2.52	111.0± 1.14
	2nd wk	274.6±6.92	279.6±5.43	272.4±4.61	277.8± 3.06
	3rd wk	587.6± 10.61 B	593.4± 11.13 B	595.2±2.57 B	621.0± 7.16 A
	4th wk	1180.2± 21.63C	1200± 19.08 B	1260.4± 5.44 A	1245.4± 5.79 A
	5th wk	1700.2± 6.30 D	1750.0± 10.00 C	1864.0±2.44 A	1830.0± 8.51 B
Body weight gain (gm)	1st wk	61.0± 0.43	64.38± 0.70	64.36± 2.77	64.10± 1.20
	2nd wk	169.4± 6.83	169.6± 5.35	161± 5.25	166.8± 3.59
	3rd wk	313.0± 14.42 B	313.8± 14.53 B	322.8± 4.06 AB	343.2± 6.46 A
	4th wk	592.6± 14.31 B	606.6± 27.84 B	665.2± 5.80 A	624.4± 8.23 B
	5th wk	520.0± 21.45 C	550.0± 23.24 B	603.6± 6.59 A	584.6± 12.08 A
Feed intake (gm)	1st wk	164.6± 2.34	164.3± 4.37	157.0± 2.95	152.7± 2.70
	2nd wk	383.5± 3.45 A	367.8± 2.20 B	327.1± 2.90 C	336.8± 2.12 C
	3rd wk	647.2± 1.72 B	657.0± 4.95 B	635.3± 5.32 C	675.2± 5.25 A
	4th wk	1205.2± 4.80 B	1212.6± 2.62 B	1232.7± 2.75 A	1204.3± 3.62 B
	5th wk	1244.1± 4.10 B	1304.9± 4.95 A	1201.3± 1.35 C	1182.5± 2.55 D
Feed conversion ratio	1st wk	2.66± 0.01 A	2.62± 0.02 A	2.41± 0.10 B	2.42± 0.04 B
	2nd wk	2.25± 0.95 A	2.16± 0.06 AB	2.02± 0.06 B	2.01± 0.04 B
	3rd wk	2.07± 0.08 A	2.09± 0.11 A	1.98± 0.02 B	1.98± 0.03 B
	4th wk	2.03± 0.05	2.01± 0.08	1.85± 0.01	1.92± 0.02
	5th wk	2.41± 0.10 A	2.39± 0.10 A	1.99± 0.02 B	2.03± 0.04 B

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

The results of (Table, 2) showed that the third and fourth group at 35 days old were increased significant (P≤0.05) in white blood cells count (28.24× 10^3±0.381 and 27.46× 10^3±0.278) as compared with the second and control group (25.76×10^3±0.212 and 22.57× 10^3±0.335) respectively. The same table showed that there was a significant (P≤0.05) decrease in Heterophil/ Lymphocyte ratio at day (35th) of the third and fourth group as compared with the second group and the control, furthermore, there was a significant (P≤0.05) decrease in Heterophil/ Lymphocyte ratio of the third treatment group as compared with the other groups at the same time. The results of the (Table, 2) showed increased in leukocytes numbers of birds which treated with embryonic vitamin E injection might be

due to the role of vitamin E in stimulation leukocytes production centers in bone marrow. As well as, vitamin E play important role in endoplasmic degeneration decreasing by reduction the harmful effects of free radicals on the cells membranes. Furthermore, decreasing in H/L ratio was evidence to the positive effect of the vitamin E in reduction the stress factors and arise immune response of the body. These results agreed with (33-35) confirmed that the importance of vitamin (E) in activation bone marrow to increase leukocytes numbers and improvement the body health status. The H/L ratio is widely used to evaluate the presence of inflammation and have been well characterized in a variety of models of avian inflammation (36). Thus, vitamin E might be attributed to health stability of treated chicks and did not expose to any stress at the study time. This was confirmed by (37) who mentioned that H /L ratio had affected by any stress factors and physiological state of chicks.

The results according to (Table, 3) showed that significant (P≤0.05) decreases in liver enzymes (AST and ALT) concentration in chicks' serum of the third and fourth treatment at aged (35) day as compared with the second and control group. As well as, there were no significant (P>0.05) differences between the third and fourth treatment at the same age.

Table, 2: Effect of vitamin E in White Blood Cells (Cell x 10^3 /mm^3) and Heterophil /Lymphocyte ratio of Blood for different groups at 35 days old. (Mean ± Standard Error).

Group / Parameter	Group 1 (Control) Inject with 0.1ml sterile PBS	Group 2 Inject with 0.1ml inactivated ND vaccine	Group 3 Inject with 0.1ml inactivated ND vaccine and 0.1ml vitamin E	Group 4 Inject with 0.1ml inactivated ND vaccine and 0.15 ml vitamin E
WBC	22.57± 0.335 C	25.76± 0.212 B	28.24± 0.381 A	27.46± 0.278 A
H/L ratio	0.84± 0.033 A	0.71± 0.004 B	0.42± 0.007 D	0.54± 0.008 C

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

Table, 3: Effect of vitamin E in biochemical parameters for different groups at 35 days old. (Mean ± Standard Error).

Group / Parameter	Group 1 (Control) Inject with 0.1ml sterile PBS	Group 2 Inject with 0.1ml inactivated ND vaccine	Group 3 Inject with 0.1ml inactivated ND vaccine and 0.1ml vitamin E	Group 4 Inject with 0.1ml inactivated ND vaccine and 0.15 ml vitamin E
AST (U/L)	230.6± 0.509 B	241.2± 1.157 A	157.2± 0.374 C	155.2± 0.583 C
ALT (U/L)	121.6± 1.077 B	152.8± 0.663 A	99.60± 1.077 C	100.4± 0.509 C
Total Protei (gm/L)	48.88± 0.333 C	53.54± 0.179 B	56.83± 0.142 A	56.21± 0.070 A
Albumin (gm/L)	23.52± 0.147 A	23.97± 0.186 A	20.30± 0.115 B	20.05± 0.160 B
Globulin (gm/L)	25.36± 0.331 C	29.57± 0.116 B	36.52± 0.173 A	36.15± 0.107 A

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

These results indicated the important role of vitamin E in regulation of liver function via preserve the liver tissue from any damages may attack by any stressor condition like in ovo vaccination, this case demonstrated by (38) who declared that enzymes AST and ALP activities were a rise in chicken serum when liver tissue damaged because of virus attack in challenge test of these chicks, whereas, increased activation of these enzymes in connection with any functional disturbances in liver cells or other organs in the body , as well as , stress condition were increased these enzymes activation in blood serum (39). However, the reduction in the AST and ALT enzyme activities were obtained of chicks were treated with vitamin E (3th and 4^{th}) groups showed a good healthy status and no functional disturbances were took place with clear improve in metabolic level, these results are agreed with (32 and 40 and 41) whom mentioned the positive effect of vitamin E in the liver, kidney and skeletal muscular tissues and other tissues of body by protecting these tissues of the progeny from oxidative injury. The results according to (Table, 3) showed that chicks total protein concentration of the third and fourth groups at (35) days of age significantly (P≤0.05) increased as compared with the second and control groups, also, the

results mentioned that significant decreases in albumin concentration and significant increases in globulin concentration at level (P≤0.05) of the third and fourth treatments as compared with the second treatment and control group. In addition, there were no significant (P>0.05) differences between the third and fourth treatments in these parameters at the age (35) days. The results of the groups which treated embryonic with vitamin E (3rd and 4th) groups showed increasing in protein concentration compared with untreated groups could be due to the role of vitamin E to prevent lipid hydro peroxide formation through its ability to quench free radicals, thus hepatic cells were protected from oxidative damage. Functionally, vitamin E protects both internal and external cellular membranes from free radical induced damage (41). The increasing in globulin concentration than Albumin concentration could be due to role of vitamin E in rising immunity response in the body by stimulate lymphocyte production which is responsible for immunoglobulins production, these results confirmed increasing in total protein concentration in blood serum samples of treated chicks with vitamin E (42). Vitamin E stimulates humoral immunity response, which mean, any increasing of B-lymphocytes lead to increasing in immunoglobulins (43 and 44) as tables (4). The researchers (45 and 46) demonstrated that vitamin E is immunoregulatory at low antigen levels; in addition, T-lymphocytes were increased in activation and proliferation because that action of immunity response led to increase cytokines production. Furthermore, these peptides (cytokines) led to increment protein biosynthesis inside the body (47 and 48).

The results of (Table, 4) showed significant (P≤0.05) differences in antibody titres against ND Virus measured by ELISA among the treatments and control group at (21 and 35) days of age, while there were no significant differences in antibody titres among groups at 7 days old of birds. The third treatment recorded that a significant (P≤0.05) increased antibody titres at 21 days old there was (2856.2±87.51) and (4712.2±154.24) at 35 days old as compared with the second, fourth and control at the same previous time. On the other hand, the fourth group recorded a significant (P≤0.05) increased antibody titres as compared with the second and control group at aged (21 and 35) days.

Table, 4: Effect of vitamin E in antibody titres against Newcastle Disease Virus measured by ELISA for different groups. (Mean ± Standard Error).

Group Age	Group 1 (Control) Inject with 0.1ml sterile PBS	Group 2 Inject with 0.1ml inactivated ND vaccine	Group 3 Inject with 0.1ml inactivated ND vaccine and 0.1ml vitamin E	Group 4 Inject with 0.1ml inactivated ND vaccine and 0.15 ml vitamin E
7 d	2678.8± 106.44	2648.6± 174.58	2651.6± 124.74	2652.2± 162.52
21 d	1668.2± 142.65 D	2446.4± 103.25 C	2856.2± 87.51 A	2795.2± 107.07 B
35 d	954.6± 44.49 D	2859.6± 122.73 C	4712.2± 154.24 A	4345.4± 177.59 B

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

The results of the current study as shown in (Tables, 4) suggested that high titer of antibodies against ND virus of the treated groups in ovo injected with vitamin E might be due to influence of vitamin E in increasing of immune response by activation and division of B-lymphocyte was led to increase antibody titres as indicated that defense of broiler chicks against ND virus. The researchers (49 and 50) confirmed that estimation antibody titres in birds' serum give good evidence to appointment immunity of birds against Newcastle disease virus. Thus, the positive results indicated that the effect of vitamin E in preservation of the immune cells and keep cellular membranes flexibility which have achieve a role in antigen diagnosis. Other authors (51) reported that humoral immune response was increased to Newcastle disease virus when broiler chicks provided at 75 IU of vitamin E/kg feed. Furthermore, in ovo vitamin E inoculation enhanced both humoral and cellular effectors components of the avian immune system (43). Also, the results of this

study agreed with (52 and 53) who referred to the importance of the use of vitamin E in alleviation of stressful situations that might bird be exposed to through a life period whereas vitamin E affects the humoral and cellular immunity.

According to the results obtained from the current study, it can concluded that in ovo injection with inactivated ND vaccine and different doses of vitamin E caused enhancement in productive traits and immune response of the hatching chicks, furthermore, the chicks were treated with 0.1 ml of vitamin E (third treatment) recorded that significant ($P \leq 0.05$) increase in mean body weight at the fifth week of age with a significant decreasing in feed consumption and improvement in feed conversion efficiency in the same time period, also showed a significant increasing in antibody titres against inactivated ND vaccine at (21 and 35) days of age as compared with other study groups.

References

1. Yegani, M. and Korver, D. R. (2008). Factor affecting intestinal health in poultry. Poult. Sci., 87: 2052–2063.
2. Foye, O.T.; Uni, Z. and Ferket, P. R. (2006). Effect of in ovo feeding egg white protein, β-hydroxy-β-methylbutyrate, and carbohydrates on glycogen status and neonatal growth of turkeys. Poult. Sci., 85:1185–1192.
3. Romao, J. M.; Vasconcelos de Moraes, T. G.; Ramos Salles, R. P.; Cardose, W. M. and Buxade, C. C. (2011). Effect of in ovo vaccination procedures on Japanese quail embryos (Coturnix Japonica) and incubation performance. J. Anim. Brasileira, 12(4):584-592.
4. Uni, Z.; Smirnov, A. and Sklan, D. (2003). Pre- and post-hatch development of goblet cells in the broiler small intestine: Effect of delayed access to feed. Poult. Sci., 82: 320–327.
5. Maija, H. Z. (1998). Vitamin A and embryonic development: an overview. J. Nut., 128(2): 455-458.
6. Bast, A.; Haenen, G. R. and Doelman, M. M. (1999). Oxidants and antioxidants: State of the art. Am. J. Med., 30(91): 2–13.
7. Applegate,T. J. and Sell, J. L. (1996). Effect of dietary linolic acid to linolenic acid ratio and vitamin E supplementation on vitamin E status of poults. Poult. Sci.,75(7): 881-890.
8. Uni, Z.; Ferket, P. R.; Tako, E. and Kedar, O. (2005). In ovo feeding improves energy status of late-term chicken embryos. Poult. Sci., 84: 764-770.
9. Boa-Amponsem, K.; Price, S. E. H.; Gerart, P. A. and Siegel, P. B. (2000). Vitamin E and immune responses of broiler pure line chickens. Poult. Sci., 79: 466-476.
10. Abdulwahid, M. T. and Al-Zuhairy, M. A. (2009). Effect of vitamin E supplement to broiler ration on physiological traits and immune response to Newcastle vaccine. Ninth Scientific Conference of Veterinary College, 24(1): 228-233.
11. Abdulwahid, M. T. and Al-Zuhairy, M. A. (2013). 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. The Iraqi J. Vet. Med., 37(2): 199 - 205.
12. Johnston, P. A.; Liu, H.; O'Connell, T.; Phelps, P.; Bland, M.; Tyczkowski, J.; Kemper, A.; Harding, T.; Avakian, A.; Haddad, E.; Whitfill, C.; Gildersleeve, R. and Ricks, C. A. (1997). Applications in ovo technology. Poult. Sci., 76: 165-178.
13. Stone, H.; Mitchell, B. and Brugh, M. (1997). In ovo vaccination of chicken embryo with experimental Newcastle disease and Avian Influenza oil-emulsion vaccines. Avian Disease. 14: 856-863.
14. National Research Council. (1994). Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press. Washington. D.C.
15. Hrubec, T. C.; Whichard, J. M.; Larsen, C.T. and Pierson, F.W. (2004). Plasma versus serum: specific differences in biochemical analytic values. J. Avian. Med. and Surg., 16: 101-105.
16. Gella, F. J.; Olivella, T.; Cruz Pastor, M.; Arenas, J.; Moreno, R. and Gomez, J. A. (1985). A simple procedure for routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. Clin. Chim. Acta., 153: 241-247.
17. Snedecor, G.W. and Cochran, W. G. (1980). Statistical methods. Iowa State University, press. Iowa.

18. Chandana, M.; Manna, S. K.; Das, R.; Batabya, K. and Roy, R. N. (2007). Development of in ovo vaccine against Newcastle disease of birds. Indian Veterinary Research Institute, Current Science. 93(9):10.

19. Cherian, G. and Sim, J. S. (1997). Egg yolk polyunsaturated fatty acids and vitamin E content alters the tocopherol status of hatched chicks. Poultry Science.76: 1753-1759.

20. Schaal, T. P. (2008). The effect of in ovo feeding of fatty acids and antioxidant on broiler chicken hatchability and chick tissue lipids. University Honors College. Oregon State University.

21. Lin, Y. F.; Chang, S. J. and Hsu, A. L. (2004). Effects of supplemental vitamin E during the laying period on the reproductive performance of Taiwan native chickens. Br. Poult. Sci., 45: 807-814.

22. Surai, P. F. (2000). Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. British Poultry Science. 41: 235–243.

23. Lin, Y. F. and Chang, S. J. (2006). Effects of dietary vitamin E on growth performance and immune response of breeder chickens. Asian-Aust. J. Anim. Sci., 19(6):884-891.

24. Surai, P.; Kostjuk, I.; Wishart, G.; MacPherson, A.; Speake, B.; Noble, R.; Ionov, I. and E. Kutz, (1998). Effect of vitamin E and selenium supplementation of cockerel diets on glutathione peroxidase activity and lipid peroxidation susceptibility in sperm, testes, and liver. Biological Trace Element Res., 64:119–132.

25. Swain, B. K.; johri, T. S. and Majumdar, S. (2000). Effects of supplementation of vitamin E, selenium and their different combination on the performance and immune response of broilers. Br. Poult. Sci., 41: 287–292.

26. Tako, E.; Ferket, P.R. and Uni, Z. (2004). Effects of in ovo feeding of carbohydrates and *β*-Hydroxy-*β*-Methylbutyrate on the Development of chicken intestine. Poult. Sci., 83: 2023-2028.

27. Uni, Z. and Ferket, P. R. (2003). Enhancement of development of oviparous species by in ovo feeding patent. North Caroline State University, Raleigh, NG. 6: 592, 878.

28. Akinleye, S. B.; Iyayi, E. A. and Afolabi, K. D. (2008). The performance, hematology and carcass traits of broilers as affected by diets supplemented with or without biomin a natural growth promoter. World J. Agric. Sci., 4(4): 467-470.

29. Dos Santos, T.T.; Corzo, A.; McDaniel, C. D.; Torres Filho, R. A. and Araujo, L. F. (2010). Influence of in ovo inoculation with various nutrients and egg size on broiler performance. J. App. Poult., 19:1-12.

30. Sahin, K.; Sahin, N. and Onderci, M. (2002). Vitamin E supplementation can alleviate negative effect of heat stress on egg production, egg quality, and digestibility of nutrients and egg yolk mineral concentrations of Japanese quails. Res. Vet. Sci., 73: 307-312.

31. Pedroso, A. A.; Chaves, L. S.; Lopes, K. L. A. M.; Leandro, N. S. M.; Café, M. B., and Stringhini, J. H. (2006). Nutrient inoculation in eggs from heavy breeders. Braz. J. Anim. Sci., 35: 2018–2026.

32. Lin, Y. F.; Tsai, H. L.; Lee, Y. P. and Chang, S. J. (2011). Maternal vitamin E supplementation affects the Antioxidant capability and oxidative status of hatching chicks J. Nut., 135: 2457–2461.

33. Whitehead, C. C.; Bollenger-Lee, S.; Mitchell, M. A. and Williams, P. E. V. (1998). The role of vitamin E in alleviating heat Stress in laying hens. Poul. Sci., 77(1): 159.

34. Morigochi, S. and Nuraga, M. (2000). Vitamin (E) and Immunity, Vitamins and Hormones. 59: 305-336.

35. Asli, M. M.; Hosseini, S. A.; Lotfollahian, H. and Shariatmadari, F. (2007). Effect of probiotics, yeast, vitamin E and C supplements on performance and immune response of laying hen during high environmental temperature. Int. J. of Poult. Sci., 6(12): 895-900.

36. Murrani, W. K.; AL-Azawi, T. S. and AL-Mossaway, A. H. (2003). Heterophil/ Lymphocyte ratio during post natal stages in chicken. IPA. J. Agric. Res., 13:1.

37. Azis, A. (2012). Performance and Heterophil to Lymphocyte (H/L) Ratio Profile of Broiler Chickens Subjected to Feeding Time Restriction. Int. J. of Poult. Sci., 11(2): 153-157.

38. Miller P. J.; king, D. J.; Alfonso, C. L. and Suarez, D. L. (2007). Antigenic differences among Newcastle disease virus strains of different genotypes used in affect viral shedding after a virulent challenge. Vaccine Aug, 3: 177.

39. Adebiyi, O. A. (2011). Tocopherol supplementation on stocking density of broiler: effect on performance characteristics and serum enzymes. Tropical and Subtropical Agroecosystems. 14: 623-628.

40. Franchini, A.; Meluzzi, A.; Bertuzzi, S. and Giordani, G. (1988). High doses of vitamin E in the broilers diets. Arch. Gef. gelk, 52(1): 12-16.

41. Arslan, M.; Ozcan, M.; Mathr, E.; Cotelioglu, U. and Ergul, E. (2001). The effects of vitamin E on some blood parameters in broilers. Turkey J. Vet. Animal. Sci., 25: 711-716.

42. Erf, G. F. and Bottje, W. G. (1996). Nutrition and immune function in chickens: benefits of dietary vitamin (E) supplementation. Pages 113–130 in: Proceedings Arkansas Nutrition Conference, University of Arkansas, Fayetteville, AR.

43. Gore, A. B. and Qureshi, M. A. (1997). Enhancement of humoral and cellular immunity by vitamin E after embryonic exposure. Poult. Sci., 76: 984–991.

44. Leshchinsky, T.V. and Klasing, K. C. (2001). Relationship Between the level of Dietary Vitamin E and the Immune Response of Broiler Chickens. Poult. Sci., 80: 1590-1599.

45. Bird, J. N., and Boren, B. (1999).Vitamin E and immunity in commercial broiler production. World Poult. Sci., 15: 20-22.

46. Puthpongsiriporn, U.; Scheideler, S. E.; Shell, J. L. and Beck, M. M. (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: 119-172, 1118-1124.

47. Erf, G. F.; Bottie, W. G.; Bersi, T. K.; Headrick, M. D. and Fritts, C. A. (1998). Effect of dietary vitamin E on the immune system in broiler, altered proportions of CD4 T- cell in the thymus and spleen. Poult. Sci., 77: 529-537

48. Boa-Amponsem, K.; Picard, M.; Blair, M. E.; Meldrum, B. and Siegel, P. B. (2006). Memory antibody responses of broiler and leghorn chickens as influenced by dietary vitamin E and route of sheep red blood cell. Administration. Poult. Sci., 85: 173-177.

49. Friedman, A.; Bartov, I. and Sklan, D. (1998). Humoral immune response impairment following excess vitamin E nutrition in the chick and turkey. Poult. Sci., 77: 956 – 962.

50. Grimes, S. E. (2002). A basic Laboratory manual for the small-scale production, and testing of 1-2 Newcastle disease vaccine. Australian center for Int. Agric. Res.

51. Nameghi, A. H.; Moghaddam, H. N.; Afshari, J. T. and Kermanshahi, H. (2007). Effect of vitamin E and C Supplementation on performance and immune response of broiler chicken. J. Anim. Vet. Adv., 6:1060-1069.

52. Weber, M.; Fodor, J.; Balogh, K.; Wagner, L.; Erdelyi, M. and Mezes, M. (2008). Effect of vitamin E supplementation on immunity against Newcastle Disease Virus in T-2 Toxin Challenged Chickens. Acta Vet. Brno. 77: 45-49.

53. Zhang, H. X.; Zhong, Y. M.; Zhou, H. M. and Wang, T. (2009). Effect of RRR-α-tocopherol succinate on the growth and immunity in broilers. Poult. Sci., 88: 959-966.

تأثير حقن بيض تفقيس فروج اللحم بجرع مختلفة من فيتامين E في بعض الصفات الإنتاجية والاستجابة المناعية للقاح مرض نيوكاسل

مشتاق طالب عبد الواحد
فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة بغداد، العراق.
E-mail: dr.m.t.abdulwahid@gmail.com

الخلاصة

أجريت التجربة لمعرفة تاثيرحقن بيض التفقيس بجرع مختلفة من فيتامين E في نسبة الفقس وبعض الصفات الفسلجية والإنتاجية والاستجابة المناعية للقاح مرض نيوكاسل. استخدمت 200 بيضة مخصبة من أمهات فروج اللحم (سلالة روز 308) وحضنت في مفقسة آلية. قسمت التجربة إلى أربعة معاملات (50 بيضة /معاملة) وكل معاملة وزعت بالتساوي وبشكل عشوائي وبواقع مكررين (25 بيضة لكل مكرر). المعاملة الاولى حقنت 0.1 مل/بيضة من محلول دارئ الفوسفات المعقم في كيس الامنيون بعمر 18 يوم من الحضن وعدت كمجموعة سيطرة. المعاملة الثانية حقنت 0.1 مل/بيضة من لقاح الزيتي المبطل لمرض نيوكاسل. المعاملة الثالثة حقنت 0.1 مل/بيضة من لقاح الزيتي المبطل لمرض نيوكاسل و 0.1 مل/بيضة من فيتامين (E). المعاملة الرابعة حقنت 0.1 مل/بيضة من لقاح الزيتي المبطل لمرض نيوكاسل و 0.15 مل/بيضة من فيتامين (E)، وبعد الإنتهاء من عملية الحقن أرجع البيض المحقون إلى المفقسة لإكمال عملية التفقيس. الافراخ الفاقسة نقلت إلى قاعة التربية في حقل دواجن كلية الطب البيطري/ جامعة بغداد حيث وزعت حسب معاملاتها السابقة لإكمال التجربة والتي استمرت لغاية الاسبوع الخامس من عمر الافراخ. أظهرت النتائج تفوق المجاميع المعاملة بفيتامين E معنويا ($P\leq0.05$) في نسبة الفقس والأداء الإنتاجي لفروج اللحم المتمثل بوزن الجسم الحي، معدل الزيادة الوزنية الأسبوعية ، استهلاك العلف الأسبوعي وكفاءة التحويل الغذائي مقارنة بالمجموعة الثانية ومجموعة السيطرة. كما اظهرت النتائج تفوق المعاملة الثالثة معنويا ($P\leq0.05$) في مستوى الأضداد المناعية في مصل دم الأفراخ ضد مرض نيوكاسل في الأيام (21 و 35) من عمر الأفراخ مقارنة بباقي معاملات التجربة ومجموعة السيطرة.

الكلمات المفتاحية: حقن بيض التفقيس، فيتامين E، الاستجابة المناعية، فروج اللحم.