ARAB JOURNAL OF NUCLEAR SCIENCES AND APPLICATIONS, 34(2) 2001
(301 - 313)

In Ovo Technology and Newcastle Disease Resistance in Japanese Quail

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(Received 21/8/2000) *(Accepted 2/12/2000)*

ABSTRACT

A total number of 1600 fertile eggs from Japanese quail birds were used in this study. The eggs were divided into eight groups then incubated. NDV and vitE were administered to the groups of eggs in Ovo injection at day 14 from incubation. Hatchability, body weight, egg production and mortality were recorded weekly for each group. Five blood samples were collected weekly from each group to measure total serum proteins, albumin, globulin, T3, T4 and HI titre. The results of this work revealed an increase in total serum proteins, globulin, and a decrease in T3, T4 and albumin values of the in Ovo vaccinating groups. Also HI titre recorded higher values due to Ovo vaccination alone or combined with vit. E. It was noticed that the group injected by inactive vaccine plus vit.E registered high increases in hatchability, body weight and egg production beside a decrease in mortality.

Key Words: In Ovo Technology / Immunity / Blood Parameters / Japanese Quail

INTRODUCTION

Studies in the last few years have indicated that live virus vaccines that are routinely administered to hatched chickens may also be injected into embryonating eggs during late stages of embryonation Sharma and Burnester[(1)] Chickens from vaccine- inoculated eggs have resistance against homologus viruses at hatching. Several commonly used vaccines, such as turkey herpes virus, infectious bronchitis virus, infectious bursal disease virus and Marek's virus may be used as embryo vaccines. Sarma[(2)] and Jamil[(3)] indicated that NDV-B1-EMS may be used as an embryo vaccine to protect chickens against Newcastle disease. Commercial use of embryo vaccination technology may reduce labor costs because semiautomatic machines with multiple injection heads that can be used to administer vaccine simultaneously into a large number of eggs Johnston[(4)] .

Vitamin E stimulates antibody response, but this does not mean that the birds are 100% disease resistant. There is unanimous agreement that administration of vit.E in excess of requirements has a stimulating effect on the production of antibodies, particularly on Immunoglobulin G (IgG) synthesis. The protective effect of vit.E may be contributed to two main factors: increase in humoral immunity and stimulation of phagocytosis Franchini[(5)] , Hoda[(6)] found that chicks vaccinated with NDV combined with vit.E showed a higher Haemagglutination inhibition (HI) titre and gave a higher protection percent compared to other different treatments. Marsh[(7)] reported that vitamin E is particularly important in the development of the immune system of the chicken. The principal objective of this work was to study the effect of applying the In Ovo vaccination with NDV vaccines alone or combined with vitamin E to the quail embryo on performance and disease resistance for post-hatched chicks.

MATERIALS AND METHODS

Fertile Eggs: A total number of 2000 Japanese quail eggs were obtained from the parent flock that is reared at the farm of the Biological Application Department of the Egyptian Atomic Energy Authority at Inshas. The eggs were collected daily for five consequtive days and stored at 15 °C. All eggs were incubated in a standard automatic incubator set at 37.8 °C and 65% relative humidity. The eggs were candled at the 13th day of the incubation period to determine the existence of growing embryo and to eliminate the dead embryos. The survive embryos (1600 eggs) were divided equally into eight experimental and control groups.

Vaccines: Live vaccine (HB1), inactivated vaccine and velogenic viscerotropic vaccine (V.V.NDV) were obtained from Veterinary Serum and Vaccine Research Institute at Abbasia, Cairo. The titre of the vaccines was 10^9 EID_{50}/1 ml. and kept at - 20 °C. till injection process.

Vitamin E (20%): It was purchased from PHENIX BELGIUM Company contained a minimum of 20% Dl-∝ m-tocopherol acetate.

Red Blood Cells: Red blood cells were centrifuged out from blood withdrawn from adult pure quails using 4% sodium citrate as anticoagulant. The red cells were washed three times with physiological saline. The centrifuged packed cells were diluted as 10% and as 0.5-1% for rapid slide haemagglutination test and quantitative haemagglutination inhibition test, respectively.

Embryo Injection: For the first 3 groups, a 100ul of saline solution contaning HB1 virus or vit.E per se or combined was injected within the allantoic region of each egg on the 14th day of incubation according to Sharma and Burnester[1], method. A 1ml syringe attached to a 1inch long 22 gauge hypodermic needle was used for injection. The entire length of the needle had to be inserted into the egg through a hole at the large edge of each egg . The hole was not sealed after injection. After completion of the injection process, the eggs were returned back to the automatic incubator for hatching stage. The same procedure was followed for the next 3 groups injected with inactivated vaccine alone, with vit.E, or with a saline solution. The last 2 experimental groups were served as control groups. The 1st control group was treated as in the commercial farms concerning the NDV vaccination and described as positive control. The 2nd control group was reared without any vaccination and served as negative control. The experimental design for the eight tested groups will be presented later on in details.

Management, Growth and Feed: The hatched quails of the different groups were battery broaded. Each group was kept in cages with dimensions of 100x80x20 cm and the temperature was settled at 37 °C during the first week then reduced by 2 °C each week until it reached 22°C by the end of the experimental period. All groups were exposed to 24 hours light during the first two weeks, then reduced by half an hour per day until it reached 16 hours daily and maintains the same during the laying period. The feed and water were supplied automatically to the birds within the batteries. Growth data were obtained by weighing (individually) the surviving quails at weekly intervals till the 7th week. At the same period, quails mortality was registered weekly. Two rations were used throughout the experimental duration:

a- Growth ration: It composed of 25% crude protein, 4% fat and 2.5% crude fiber. This ration contained 3400 /Kcal-ME/kg diet.

b- Laying ration: It contained 17% crude protein, 1.5% fat, 3% crude fiber and contained 3100/Kcal-ME/kg diet. Vitamins, minerals and feed additives were also added to both rations according to the recommended programs.

Egg Production: At the onest of first laying egg, 25 females from each treatment were kept in laying batteries. Egg production was recorded daily for each treatment. The quail day production (No. of

eggs/No. of females x100) was calculated in weekly order for 10 weeks from the 1 st day laying of each treatment.

Blood Samples: Five birds representing the average body weight were selected from each treatment weekly and along 7 weeks period. At the 1st, 2nd and 3rd weeks, the blood samples were withdrawn after decapitation, while at the 4 th,5 th,6 th and 7 th weeks the blood samples were drawn from the wing vein. The individual blood samples was collected in a siliconized tube containing heparin, and plasma was separated by centrifugation then stored at -20 °C until analysis.

Total Plasma Proteins: It was determined using the Biuret method described by Armstrong and Carr[8] . Serum albumin was measured by using bromocresolgreen (BCG) after the method of Doumas[9]. Serum globulins were calculated by substracting serum albumin from serum total proteins for each quail.

Thyroid Hormones (T_3 and T_4) : Total thyroxin (T_4) and triiodothyronine (T_3) were assayed in quail serum by radioimmunoassay (RIA) techniques based on the methods described by Chopra[10], Larsen[11] , using kits purchased from Clin. Lab. Specific anti T3 and T4 sera raised in rabbits were obtained from diagnostics Biochem. Canada Inc. London, Ontario, Canada. Labelled hormones (I^{125} T3 and I^{125} T4) were prepared in the Atomic Energy Authority at Inshas. Purification of the iodinated T3 and T4 was carried out according to the method described by Ibrahim[12] .

Haemagglutination Inhibition (HI) Test: The test was carried out according to the standard procedure described by Majijabe and Hitchner[13].The titration of the haemagglutination activity of the NDV antigen was an essential primary procedure using the HA test, Anon[14] in order to determine the number of haemagglutination HA units of the virus to be used in the haemagglutination inhibition proper test.

Protection(Challenge)Test:-A random samples of 10 birds from each group were inoculated with 0.1ml of (V.V.NDV) having a titre of $10^{5.5}$ LD_{50} at 20 and 40 day of age. The quail mortality was registered daily along an observation time of 10 days.

Experimental Design: Eight groups of survived embryonated quail eggs(14 days old) were classified as follows:

Group 1(G1): was injected with vitamin E only (10 mg vitamin E particles in 100 µl saline /embryo).

Group 2(G2): was inoculated with vit. E and live HB1 ND vaccine(10 mg vit.E +250000 virus particles in 100 µl saline /embryo).

Group 3(G3): was inoculated with vit. E and inactivated ND vaccine (10mg vit. E in 100µl inactivated ND vaccine/embryo).

Group 4(G4): was inoculated with live HB1 ND vaccine only (250000 virus particles in 100µl saline / embryo).

Group 5 (G5): was inoculated with inactivated ND vaccine only (100 µl / embryo).

Group 6 (G6): was injected with saline solution 0.9% (100 µl / embryo).

Group 7(G7): The hatched quails in group 7 (+ve control) were vaccinated against Newcastle Disease at the 5th day of age by dipping using Hitchner B1 strain and at 21 th, 40th and 60 th days of life span using Lasota strain.

Group 8(G8): Both embryonated and hatched quails of this group were kept without any treatments (-ve control).Statistical analysis: The data were analyzed by ANOVA in one -way classification using the General Linear Models (GLM) procedure according to Snedecor and Cochran[15].

RESULTS AND DISCUSSION

Hatchability, Hatching Weight and Quail Mortality:

Hatchability percent, hatching weight in grams and total quail mortality percent are presented in Table (1). The results indicated that inoculation of quail embryos with vit.E and inactivated NDV vaccine (G3), gave the highest hatchability percentage. This positive effect did not occur when the vitamin E was injected alone (G1) or combined with live Hitchner B1 vaccine(G2). At the same incubation time, applying Hitchner B1 or the inactivated vaccines alone (G 4 and G5) recorded higher hatchability values comparing with (G6) which received saline only and (G7andG8) the +ve and -ve controls, respectively. No different variations were obtained between the experimental groups concerning hatching weight, while the total quail mortality during the first seven weeks calculated from the hatched quails showed an observable variations. It was noticed that introducing vit.E with the inactivated vaccine (G3) to the quail embryos reduced the total post hatch quail mortality to be 1.3 % comparing to a value of 8.9% in the negative control group (G8). On the opposite, inoculating the survival quail embryos with live HB1 vaccine alone (G4) or combined with vit.E (G2) resulted in an obvious increase in total mortality being 13.1 and 10.0%, respectively. Vaccinating the embryos with the inactivated vaccine (G5) gave a good low total quail mortality of 2.0%, while injecting vit.E alone (G1) gave an intermediate value, 6.7%, between those of the negative and positive control (G7 and G8) ratios.

Most of the scientists concentrated their researches on the relation between vit. E addition and increasing immunity Haq[16] or to the transfer of the antidiseases due to embryo vaccination to the hatched birds Ahmad and Sharma[17]; Radwan[18] and Johnston[4]. Indeed, the successful immunization of chickens before hatching and producing immunized chicks beside the effect of the In Ovo injections on hatchability, post-hatch mortalities and growth rate should be taken into consideration. The improvement in hatchability percent in this study, following the inoculation of NDV vaccines (G3, G4 and G5) during the last embryonating days is considered good results and ascertained that obtained by Gore and Quzeshi[19]. The results of the abovementioned treatments demonstrated the superiority of them over the sham and control treatments in hatchability process.

The lowest total quail post-hatch mortality herein was achieved due to inoculation of the inactivated NDV alone or incorporated with vit.E during the last incubation interval. It is a well known phenomena that vit.E is an effective stimulant to immune systems in chickens. The passively transferred antibody levels are significantly increased in plasma of young chicks when hens were fed high levels of vit.E, El-Boushy[20]. Moreover, the protective effect of vit.E may be contributed to the increase in humoral immunity and stimulation of phagocytosis. The abovementioned data cleared also that this route of vaccination was succeeded to protect the birds against the dangerous infection and was enhanced by the combination with vit.E. In this respect, McL l roy[21], suggested that, the improvement in performance from vit.E addition may be due to enhanced immunocompetence and increased resistance to disease. Furthermore, Franchini[22], recorded that vaccines with vit.E induce more rapid and higher antibody response than control vaccines. On the other hand, injecting quail embryos with live Hitchner ND vaccine alone or combined with vit.E failed to proceed the same protection to the hatched birds as with the inactivated vaccine. That may be due to the sensitivity of the injected embryos to the different types of ND vaccines or to the varied interaction with vit.E. At the same time, inoculation of vit.E alone to the embryos did not produced the predicted effect on mortality rate since it is proved that vit.E is most beneficial where there is a challenge to the host defense system,McL l roy[21].

Table (1) Hatchability percent, total quail mortality and hatching weight in the different treated groups.

Parameters	Groups							
	G1	G2	G3	G4	G5	G6	G7	G8
Hatchability%	83.0	66.0	99.0	94.0	94.8	91.5	93.0	92.3
Total quail mortality%	6.70	10.00	1.30	13.10	2.00	3.50	5.80	8.90
Hatching weight(g)	8.80	8.64	8.60	8.63	8.80	8.86	8.69	8.94

Growth and Egg Production:

The changes in body weight data during the seven weeks post hatching are presented in Table (2). It was noticed that the significant increase ($P<0.05$) in quail body weight during the first 2 post hatch weeks was recorded for G5, that was injected by the inactivated vaccine alone as embryos whereas G1, G2 and G3, that were inoculated respectively with vit.E alone or combined with Hitchner (HB1) or inactivated vaccine, registered the significant increase in body weight during the following five consecutive weeks. The retardation in growth rate was observed in the weights of the sham and control groups (G6, G7 and G8).

The situation concerning egg production data during a production period of ten weeks was somewhat different somewhat than growth rate as illustrated in Table (3). The increase and stability in quail/day production was much clearly achieved in G3 that received vit.E and inactivated vaccine as compared with the positive control group (G7). On the contrary, applying HB1 live vaccine (G4) to the quail embryos resulted in a considerable decrease in egg production during the experimental laying interval.

It seems that the early vaccination during the late embryonic stage against ND, especially when connected with vit.E inoculation, produced healthy baby quails and enhanced the components of the immune system. There is evidence that increasing the intake of vit.E improves the health status and disease resistance of poultry, Jerry[23]. The last authors found that diets containing 6 to 20 IU of vit.E supported satisfactory growth, feed efficiency and livability of tom turkeys from 1d of age to market age. In this respect, Bollengier ,[24] pointed out that the overall effect with the vit.E treatment was an improvement in FCE which represents an additional commercial advantage to the use of vit.E especially under stress. In another survey, McIlroy[21], demonstrated that the results of feeding broiler flocks on high vit.E containing diet was 8.2% ($P<0.01$) better than that achieved by flocks being fed on normal diets.

On the other hand, the improvement in the health status of the hatched quails could be related to the increase in antibody molecules. The chicks hatched from hens supplemented with vit.E had significantly higher antibody titers at 1 and 7 d of age than chicks from control group Haq[16]. The enhancement of antibody production and the increase in the immune response of the progeny due to vit.E supplementation of breeder birds were mentioned by Eric, Gershwin[25]. Therefore, it is recommended to increase vit.E levels at hatch via In Ovo injection of embryos 3d before hatch since it is a time when the bird is faced with environmental challenges of stress and disease-causing organisms Gore and Qureshi[19].

The increase and stability in egg production due to the inoculation of vit.E with the inactivated NDV vaccine in the current study reflects the beneficial effect of this treatment. It was suggested that high dietary inputs of vit.E are most beneficial where there is a challenge to the host defense system and significantly improved performance will occur more predictably, McIlory[21]. The last author added that vit.E functions primarily as a lipophilic antioxidant capable of preventing free radical-medicated lipid peroxidation within membranes. In this respect, Bollengier ,[24] mentioned that there are 2 mechanisms, protection of the liver or other organs against oxidative damage and

restoration of oestrogen secretion could, independently or together, explain the effects of high concentrations of vit.E on the egg yolk precursor economy and improved egg production in laying hens.

Table (2) Body weight changes (Mean in Grams in the different treated groups)

Weeks	Groups							
	G1	G2	G3	G4	G5	G6	G7	G8
1stWeek	24.9^{a} ±3.5	20.0^{e} ±1.8	23.2^{c} ±1.7	22.4^{d} ±1.6	25.1^{a} ±1.2	23.8^{b} ±1.5	24.3^{b} ±1.8	23.2^{c} ±1.4
2nd Week	46.8^{c} ±3.3	44.6^{d} ±4.4	48.8^{b} ±3.2	45.4^{d} ±4.3	50.8^{a} ±3.6	50.6^{a} ±3.8	47.9^{b} ±4.3	47.9^{bc} ±4.7
3rdWeek	88.7^{b}± 9.1	91.3^{a} ±7.1	91.8^{a} ±4.9	89.9^{ab} ±9.2	90.8^{a} ±5.0	86.6^{b} ±5.3	84.2^{c} ±7.1	79.2^{d} ±5.1
4th Week	140.6^{a} ±13.9	140.4^{a} ±12.5	142.3^{a} ±6.8	129.5^{c} ±15.2	139.2^{ab} ±9.2	136.7^{b} ±10.5	134.6^{b} ±13.4	128.4^{c} ±7.8
5th Week	183.2^{b} ±16.7	188.9^{a} ±20.1	185.2^{a} ±19.4	180.7^{b} ±17.6	182.3^{b} ±17.5	179.3^{b} ±17.9	180.1^{b} ±17.9	174.9^{b} ±18.9
6th Week	224.1^{a} ±26.9	218.2^{a} ±24.5	215.0^{b} ±30.8	212.9^{b} ±22.3	208.7^{b} ±29.0	208.9^{b} ±28.8	209.6^{b} ±29.1	205.0^{b} ±25.5
7th Weeks	234.9^{a} ±30.3	232.9^{a} ±26.4	228.2^{a} ±32.6	218.8^{b} ±29.1	213.9^{b} ±31.8	221.6^{b} ±31.2	215.7^{b} ±24.8	214.4^{b} ±34.3

a,b,c,.. means in the same row with different superscripts are significantly different ($p<0.05$).

Table (3) Egg Production data (Q.D.%) in the different treated groups

Weeks	Groups							
	G1	G2	G3	G4	G5	G6	G7	G8
1stWeek	9.7	12.4	7.8	6.0	4.7	1.7	7.5	6.2
2nd Week	72.8	60.0	68.3	46.6	56.9	39.0	54.6	56.5
3rd Week	83.4	79.3	88.6	53.1	84.0	78.9	80.0	80.0
4th Week	84.5	72.1	86.3	52.0	83.4	77.9	85.1	84.6
5th Week	80.1	65.4	85.7	54.8	77.1	80.9	82.3	80.0
6th Week	81.4	66.2	80.0	53.8	82.3	80.9	86.9	84.6
7th Week	68.3	60.9	79.4	52.9	74.9	73.8	84.5	77.1
8thWeek	77.9	53.8	81.1	42.0	73 7	76.8	84.6	83.3
9thWeek	77.3	76.5	82.1	69.7	72.6	67.8	80.1	75.0
10th Week	74.0	74.7	80.3	61.3	77.3	62.5	85.3	58.4

Blood Serum Proteins:

It was indicated from the results of total serum proteins (Table 4) that the inoculated groups with vit.E and NDV alone or combined (Groups 1 to 5), registered the highly significant increase values along the experimental period. At the same time, the negative control group (G8) showed the lowest significant decrease values in most tested weeks, while the positive control and saline injected groups (G6 and G7) gave an intermediate results.

It seems likely that the increase in the total serum proteins observed in the inoculated groups (G1 to G5) is referred to the significant decrease in serum albumin values (Table 5) and to the significant increase in serum globulins (Table 6) as compared to the corresponding values of the un-vaccinated group (G8) .

The observed results on serum proteins herein agreed well with that obtained by Abdel Messih[26], who noticed a significant increase in the level of total serum proteins of the Hubbard chicks vaccinated with Newcastle disease virus. He found the highest increase was at 4weeks post vaccination and attributed it to the increase in the level of immunoglobulin IgG and total globulins especially the Gamma-fraction which contains most of the antibody activity. The same opinion was described by Rivetz[17], who referred the increase in serum total proteins to an enhanced synthesis of immunoglobulins and a large increase in the level of Alpha-glycoproteins, probably due to its release from tissues as a part of the inflammatory response.

On the other hand, the significant reduction in serum albumin occurred in the vaccinated quails of this work, could be due to general response of body to vaccine. Such relative decrease might likely be return to reduced feed intake, or to mobilization of albumin to meet hypoglycemia. Besides, albumin is believed to act as a protein reserve and a protein source for amino acids at times of subnormal intake of food Sturkie[28]. This fall in serum albumin was manifested also by Abdel Messih[25] and Bertil-Laurell[29] who revealed that decrease to the shift of albumin to increased synthesis of protective proteins particularly stress proteins or acute phase proteins. So the significant increase in globulin fraction happened in this study could confirm this suggestion.

The significant increase in globulin fraction noticed here could be due to its enhanced synthesis by the defense system of the body to face the early embryonic vaccination process. This increase in serum total globulins of vaccinated groups might therefore be due to vaccine application which began to stimulate immune system, consequently antibodies were produced, Rivetz[27]. In this respect, Deluca[30] concluded that the increase in the levels of immunoglobulin, post vaccination with Newcastle disease virus vaccine, could be referred to alteration in epithelial structure, that normally provide a barrier to entry for infectious agents.

Table (4) Mean values of total Serum Proteins (g %) in the different treated groups

Weeks	Groups							
	G1	G2	G3	G4	G5	G6	G7	G8
1st Week	3.56±.31	3.71±.55	3.39±.36	3.50±.77	3.61±.35	3.37±.39	3.44±.17	3.06±.13
2nd Week	3.41^{c}±.69	3.55^{bc} ±.41	4.06^{a}±.25	3.36^{c} ±.38	3.89^{ab} ±.18	4.07^{a}±.49	3.59^{bc} ±.39	3.74^{b} ±.12
3rd Week	3.81^{b}±.50	3.81^{b} ±.51	3.71^{b} ±.45	3.69^{b} ±.28	4.37^{a}±.25	3.67^{b}±.34	4.29^{a}±.57	3.56^{b} ±.25
4th Week	3.66^{ab} ±.59	3.65^{ab} ±.33	4.27^{a}±.53	3.97^{a}±.61	3.77^{a}±.55	3.63^{ab} ±.24	3.38^{ab} ±.34	3.23^{b} ±.63
5th Week	3.71±.45	3.66±.69	3.81±.55	4.33±.29	3.71±.59	3.65±.59	4.15±.61	3.42±.28
6th Week	4.10^{a}±.62	3.35^{c} ±.24	3.64^{bc} ±.23	3.49^{bc} ±.28	4.22^{a}±.43	4.12^{a}±.76	3.40^{c} ±.65	3.78^{b} ±.45
7th Week	3.80^{ab} ±.45	3.65^{b} ±.45	4.19^{a}± 43	3.78^{ab} ±.43	3.72^{ab} ±.43	3.51^{b} ±.32	4.56^{a}±.63	3.41^{b} ±.79

a,b,c,.. means in the same row with different superscripts are significantly different ($p<0.05$).

Table (5) Mean values of Serum Albumin (g %) in the different groups.

Weeks	Groups							
	G1	G2	G1	G4	G5	G6	G7	G8
1st Week	1.38±.20	1.52±.16	1.29±.12	1.33±.43	1.47±.24	1.29±.19	1.51±.14	1.50±.08
2nd Week	1.41^{d}±.13	1.48^{d} ±.21	1.84±.56	1.65^{c} ±.23	1.81±.11	1.68^{c} ±.39	1.75^{bc} ±.21	1.89^{a} ±.08
3rd Week	1.53^{c} ±.25	1.76^{b}±.24	1.66^{b} ±.17	1.62^{b} ±.27	1.73^{b} ±.18	1.67^{b} ±.28	1.95^{ab} ±.20	2.19^{a} ±.19
4th Week	1.73±.32	1.68±.42	1.39±.26	2.04±.27	1.81±.35	1.72±.40	1.67±.34	1.93±.01
5th Week	1.53±.25	1.35±.18	1.70±.26	1.76±.33	1.59±.37	1.55±.20	1.74±.29	1.66±.15
6th Week	1.60^{d} ±.34	1.80^{c} ±.08	1.80^{c} ±.28	1.79^{c} ±.25	1.81^{c}±.12	1.92^{b} ±.18	1.99^{ab} ±.14	2.13^{a} ±.20
7th Week	1.02±.12	1.24±.42	1.49±.33	1.23±.38	1.67±.34	1.38±.21	1.49±.43	1.60±.47

a,b,c,.. means in the same row with different superscripts are significantly different (p<0.05).

Table (6) Mean values of Total Serum Globulin (g %) in the different groups.

Weeks	Groups							
	G1	G2	G3	G4	G5	G6	G7	G8
1stWeek	2.18±.48	2.19±.62	2.10±.43	2.17±.72	2.14±.50	2.07±.51	1.93±.14	1.56±.13
2ndWeek	1.99±.56	2.07±.56	2.22±.19	1.72±.45	2.09±.18	2.39±.48	1.80±.31	1.85±.19
3rd Week	2.27^{a}±.69	2.05^{ab} ±.50	2.05^{ab} ±.56	2.08^{ab} ±.51	2.64^{a} ±.27	2.00^{ab} ±.49	2.34^{a} ±.49	1.37^{b} ±.25
4th Week	1.93^{b} ±.44	1.97^{b} ±.29	2.88^{a} ±1.20	1.93^{b} ±.38	1.95^{b} ±.39	1.91^{b} ±.47	1.71^{bc} ±.57	1.30^{c} ±.60
5th Week	2.18±.89	2.30±.75	2.11±.68	2.57±.77	2.11±.35	2.11±.68	2.41±.56	1.76±.84
6th Week	2.50^{a} ±79	1.95^{b} ±.21	1.84^{b} ±.39	1.71^{bc} ±.47	2.41^{a} ±.40	2.23^{ab} ±.66	1.41^{c} ±.58	1.65^{bc} ±.44
7th Week	2.78±.41	2.41±.66	2.74±.55	2.55±.68	2.05±.23	2.13±.46	2.20±.97	1.81±.39

a,b,c,.. means in the same row with different superscripts are significantly different (p<0.05).

Thyroid Hormones:

The concentration of both of T3 (3,5,3 triiodothyronine) and T4 (3,5,3,5 tetraiodothyronine) in the quail serum of the different experimented groups along the growth period, 1 to 7 weeks of age, are illustrated in Tables (7 and 8). It was obvious that, most of the significant effect for both parameters was recorded in the negative control (G8). In other words the un-vaccinated,un-inoculated group (G8) registered the highest values for T3 and T4 along most of the experimental period. On the other hand, inoculating vit.E alone or combined with NDV strains to the quail embryos at the last embryonating stage resulted in a significant ($P<0.05$) reduction in T3 and T4 concentrations during several post-hatch weeks. The data of the two tested hormones in the control group agree well with that mentioned by Wakwak[31] on the same breed.

Very rare information are available concerning the relation between NDV vaccination or vit.E inoculation and thyroid gland function. However, there is evidence that T4 concentrations were higher and body weight was lower in japanese quail between 0 to 4 weeks of age David[32]. That finding may explain the results observed in this study, since T4 concentration was decreased and body weight was increased due to injecting quail embryos in Ovo with vit.E and/or NDV vaccines. Moreover, Bachman and Mashaly[33] mentioned that, T3 supplementation reduces relative thymus and spleen weights and hence decreases total circulating white blood cells and number of lymphocytes in male chickens.

Therefore, the reduction achieved in T3 values herein could be responsible for enhancing immunostimulatory system. As mentioned before globulin fraction, that portion of total proteins concerned with immuity, was significantly increased in the groups inoculated, as embryos, with vit.E and NDV vaccines.

Table (7) Mean ±(S.E) concentration of T3(µg%)in the different treated groups

Weeks	Groups							
	G1	G2	G3	G4	G5	G6	G7	G8
1 st Week	120.3^{d}±0.6	128.2^{d}±1.8	126.8^{b}±10.2	128.6^{b}±2.0	125.3^{c}±5.8	128.8^{b}±2.6	127.4^{d}±1.5	132.8^{a}±7.5
2 nd Week	180.5±25.9	124.1±11.2	125.7±5.4	131.2±5.5	120.5±4.4	128.6±5.3	128.5±23.8	128.0±10.3
3 rd Week	204.2^{a}±36.2	139.6^{c} ±7.7	136.9^{c} ±30.5	126.3^{d} ±7.6	148.5^{c} ±9.7	143.6^{c}±15.2	136.5^{c} ±14.2	182.7^{b} ±20.1
4 th Week	135.7^{d}±20.3	119.6^{d} ±9.4	156.1^{c} ±17.5	174.7^{b} ±40.5	193.3^{a} ±38.1	140.5^{c} ±35.3	186.1^{b} ±36.8	205.1^{a} ±14.2
5 th Week	138.9±13.6	150.9±27.1	166.1±38.1	153±45.5	184.7±71.5	211.2±49.7	168.4±28.1	180.1±23.7
6 th Week	128.6^{d}±8.6	135.3^{d}±9.2	162.4^{b}±22.2	174.7^{a}±15.8	176.5^{a}±7.3	151.1^{c}±6.8	121.5^{d}±17.1	180.9^{a}±9.3
7 th Week	134.4^{c}±24.1	145.2^{c}±21.1	145.7^{c}±9.9	194.4^{a}±48.1	170.3^{b}±161	163.6^{b}±17.9	163^{b}±19.6	177.2^{b}±20 1

a,b,c,.. means in the same row with different superscripts are significantly different (p<0.05).

Table (8)Mean ±(S.E) concentration of T4(µg%)in the different treated groups

Weeks	Groups							
	G1	G2	´G3	G4	G5	G6	G7	G8
1stWeek	6.1^{b}±1.1	7.1^{a}±0.6	7.4^{a}±0.7	6.5^{b}±1.2	5.2^{c}±0.6	7.2^{a}±0.6	6.4^{b}±0.5	7.0^{a}±1.3
2ndWeek	6.9^{b}±1.3	6.3^{b}±1.2	5.6^{c}±1.3	7.6^{a}±0.8	5.4^{c}±1.3	5.5^{c}±1.4	5.9^{c}±.42	6.4^{b}±0.5
3rdWeek	8.9±0.5	7.6±0.9	6.7±0.7	7.7±0.5	7.6±1.2	8.0±1.8	8.1±.82	8.8±1.3
4thWeek	8.4^{bc}±0.8	8.0^{c}±1.5	8.6^{bc}±0.6	7.8^{c}±0.7	9.1^{b}±0.7	9.5^{b}±1.6	8.8^{bc}±0.4	10.2^{a}±0.3
5thWeek	7.7± 1.4	7.7±1.1	7.4±1.1	9.8±.67	9.0±1.9	8.9±1.0	9.3±3.3	10.9±2.7
6thWeek	7.3^{c}± 1.3	7.5^{c}±0.5	8.1^{c}±0.5	9.8^{a}±2.5	10.3^{a}±2.3	9.6^{a}±2.2	9.4^{b}±1.6	10.6^{a}±2.1
7thWeek	7.6^{b}± 1.1	7.5^{b}±1.8	8.5^{b}±1.5	10.6^{a}±3.5	8.8^{b}±2.6	10.1^{a}±.87	11.1^{a}±2.3	10.7^{a}±2.7

a,b,c,.. means in the same row with different superscripts are significantly different (p<0.05).

Haemagglutination Inhibition Test (HI) and Trotection Test:

HI test is considered one of the best methods for assaying the amount of the antibody existed in the host serum quantitatively against known disease. The In Ovo vaccinated groups, G2 to G5, recorded higher values for HI titre at weeks 1,3,4 and 6 as compared with the control group,G7, as illustrated in Table9. On the contrary, control, G7, group that was vaccinated against ND 3 times post-hatch during the seven growth period weeks showed the highest HI titre after each vaccination. This result may demonstrate that In Ovo vaccination could acquire protection and provide enough antibody production for the post-hatch quails against ND, especially during the growth stage.

Applying the protection (challenge) test on the different experimented groups cleared that a hundred protection percent was achieved in the vaccinated groups, whether embryonically or post-hatch, at 20 days of age (Table 10). This value of protection was registered to be zero in the un-vaccinated groups. At 40 days of age the ratio of protection in the in Ovo vaccinated groups ranged between 70 and 90 percent, while it was zero percent in the control group. At the same time, the low

protection values, 10 to 30 percent, obtained in the un-vaccinated groups could be referred to the naturally acquired protection.

Considerable evidence has been presented showing that supplemental dietary vit.E enhances immune competence and increases humoral immune response and antibody titers against diseases in chickens Meydani[34]. The influence of supplemental vit.E on immune competence of chickens may be mediated through changes in prostaglandin synthesis in the bursa of fabricius and spleen, Lawrence[35], while the increase in humoral immune response and haemagglutination inhibition titers indicates that vit.E acted specifically at the level of the helper T cell Nockels[36]. It was mentioned also that, chicks from hens receiving vit.E had significantly higher antibody titers at 1 to 7 days of age Haq[16]. Moreover, they added that, supplemental vit.E, when fed to breeder birds, increases the humoral immunity of their progeny as measured by antibody production against NDV of 2 and 3 weeks old chickens. In addition, the NDV vaccine response was enhanced in chicks given 10 to 30% vit.E-added vaccine as compared with those given vaccine emulsified in light mineral oil only Gore and Qureshi[19]. All of those studies agree well with the results of this work and emphasize the beneficial role of inoculating vit.E with NDV vaccines In Ovo on improving immunity process and disease protection.

In conclusion, In Ovo inoculation of vit.E with NDV vaccines, especially the inactive strain, produced healthy immunized baby quails, improved the normal growth rate and egg production capability and provided the immunity needed for protection from ND. Therefore, from a practical standpoint, the In Ovo route may currently be used for delivering such vaccine and by providing vit.E access to the bird embryos, in addition to the vit.E made available through diet, the immune enhancement benefits of vit.E may be better realized soon after posthatch.

Table (9) Mean detection of Log 2 HI titer in the different treated groups

Weeks	Groups							
	G1	G2	G3	G4	G5	G6	G7	G8
1 st Week	–	6.8	6.2	7.2	6.8	–	–	–
2nd Week	–	7.2	6.6	8.4	7.6	–	10.2	–
3rd Week	–	9.6	8.4	9.8	9.2	–	4.8	–
4th Week	–	9.4	8.6	9.0	6.2	–	5.4	–
5th Week	–	6.8	7.0	6.6	6.0	–	9.4	–
6th Week	–	6.0	6.7	5.8	5.2	–	5.2	–
7th Week	–	5.7	5.4	5.2	5.0	–	6.7	–

Table (10): Result, of Protection test in the different treated groups

Groups	Challenge Time Days	No of quails	Death	Alive	Protection
G1	20	10	10	--	00
G2		10	--	10	100
G3		10	--	10	100
G4		10	--	10	100
G5		10	--	10	100
G6		10	10	--	00
G7		10	--	10	100
G8		10	10	--	00
G1	40	10	8	2	20
G2		10	1	9	90
G3		10	1	9	90
G4		10	3	7	70
G5		10	3	7	70
G6		10	7	3	30
G7		10	--	10	100
G8		10	9	1	10

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Arab Journal of Nuclear Sciences and Applications, 34(2), (301 - 313) 2001

تكنولوجيا حقن الأجنه ومقاومه مرض النيوكاسيل فى السمان اليابانى

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خلاصة البحث

يعتبر مرض النيوكاسيل من اهم واخطر الا مراض الفيروسيه التى تهدد انتاج الدواجن فى كثير من بلاد العالم وخاصة الشرق الأوسط ومصر حيث انه يسبب معدل وفيات عاليه جدا فى الطيور المصابه بالمرض علاوه على انخفاض معدل النمو وانتاج البيض

وتشير الأبحاث أن فيتامين هـ من الفيتامينات التى تعمل على زياده الانتاج والخصوبه والمناعه. فكان الهدف من هذه الدراسه استخدام تكنولوجيا حقن الاجنه بفيرض النيوكاسيل وفيتامين هـ منفرده أو مجتمعه فى أجنه طيور السمان اليابانى عند عمر 14 يوم وتقييم المناعه المكتسبه لدى الطيور الفاقسه حتى عمر التسويق ضد مرض النيوكاسيل. كما تم دراسه اثر عمليات الحقن المختلفه على معدلات النمو والفقس وانتاج البيض وقياس البروتين الكلى والالبيومين والجلوبيولين فى الدم وقياس هرمون الغده الدرقيه (الثيروكسين وترای ايودو ثيرونين) باستخدام طرق المناعه الاشعاعيه.
واستخدمت فى هذه الدراسه 1600 بيضه مخصبه وقسمت الى 8 مجاميع كم يلى.
1- المجموعه الاولى تم الحقن بفيتامين هـ فقط.
2- المجموعه الثانيه تم الحقن بفيتامين هـ مع لقاح هتشنر حى .
3- المجموعه الثالثه تم الحقن بفيتامين هـ مع لقاح ميت .
4-المجموعه الرابعه تم الحقن بلقاح هتشنر حى .
5-المجموعه الخامسه تم الحقن بلقاح هتشنر ميت .
6-المجموعه السادسه تم الحقن بمحلول ملحى .
7-المجموعه السابعه كونترول وتم تحصين السمان عند عمر 5, 21, 40, 60 يوم .
8-المجموعه الثامنه كونترول عام لم يتم الحقن او التحصين.
وتشير النتائج المتحصل عليها الى امكانيه استخدام هذه التكنولوجيا الحديثه بنجاح فى التحصين ضد مرض النيوكاسيل خلال مرحله الاجنه كما اتضح من زياده المناعه المكتسبه ضده عن الكونترول العام وزياده نسبه البروتين والجلوبيولين فى الدم ونقص فى نسبه الالبيومين وهرمون الثيروكسين وترای ايودو ثيرونين.
لوحظ ايضا تفوق المجموعه التى تم حقن الاجنه باللقاح الميت مع فيتامين هـ عملت على رفع نسبه الفقس وزياده فى وزن الجسم وانتاج البيض وانخفاض النفوق عن باقى المجاميع.