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# EFFECT OF VITAMIN E AND SELENIUM ON THE IMMUNE RESPONSE OF CHICKENS AGAINST LIVING NEWCASTLE DISEASE VACCINE.

BY

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## INTRODUCTION

Nutrition-immune response interaction is a complex relationship. The cell-mediated and humoral immunity are severly depressed in acute or chronic malnutrition. This has been emphasised by Sheffy and Williams (1982). It is now well established that deficiency of vitamin E and selenium has a significant suppressive effect on the immune system, particularly, cell - mediated mechanism (Sheffy and Schultz, 1979; Marsh et al., 1981). On the other hand, dietary supplementation with vitamin E and selenium at levels above the established requirements stimulate the primary immune response (Spallholz et al., 1973; Sheffy and Schultz, 1977 & 1979; Marsh et al., 1981; Colnago et al.,1984). More over, it has been reported that a high dose of dietary vitamin E improved significantly the body weight gain (Heizerling et al., 1974; Colnago et al., 1984; Farouk, 1988; Zaki et al., 1989).

In Egypt, NewCastle disease (ND) is considered as one of the most dangerous viral disease that infects chickens which could be controled by various vaccination processes. Law (1976) and Farouk (1988) recorded a double haemagglutination inhibition ( HI )

antibody titres against ND virus in dietary vitamin E supplemented chicks two weeks psot-vaccination with La Sota strain. On the other hand, Bassiouni et al. (1985) stated that clinical and immunological status of ND were not affected by tocopherol serum level.

Therefore, the aim of the present study was to detect the possible effect of vitamin E and selenium ( Se ) supplementation on the immune response of chicks vaccianted with living ND vaccines as measured by HI antibody titres and protection against challende with velogenic ND virus as well as on the body weight and feed conversion efficiency.

## MATERIALS AND METHODS

**Experimental birds:** One hundred and twenty five one-day old male L.S.L. chicks (layer strain), were allocated into five groups (1-5). Each group was housed separately under 24 hours light schedule.

**Diet:** All group were fed a basal commercial diet composed of 66% group yellow corn, 24% soyabean meal and 10% concentrate mixture (50% crude protein). The diet furnished 21.4% crude protein, 3.5% fat and 3.4% crude fiber. The calculated analysis of vitamin E and selenium was 27.24 and 0.15 mg.kg diet respectively, which covered the bird requirements. Vitamin E and selenium were added to the basal diet at level of 300 mg/kg (Tengerdy and Brown, 1977; Franchini et al., (1983) and 0.25 mg/kg (Colnago et al., 1984) respectivly, two weeks before vaccination with La Sota. Vitamin E added was in form of DL-alpha tocopherol acetate, while selenium was added in form of sodium selenite. Different treatments for each experimental group are illustrated in **(Table, 1)**.

Table, 1: Different treatments of the experimental groups for 8-weeks experimental period.

| Group No. / Age in days. | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 0 - 7 | B.D.* | B.D | B.D | B.D | B.D |
| 8 - 21 | B.D + 300 mg/kg vit. E | B.D + 300 mg/kg vit. E and 0.25 mg.kg Se. | B.D + 0.25 mg.kg Se | B.D | B.D |
| 22-52 | B.D | B.D | B.D | B.D | B.D |

Group 4: Non-treated vaccinated control.
Group 5: Non- treated novaccinated control.

The basal diet contained:
(Bit A 120.000 1.4., $D_3$ 25 ouo Iu, vit E 100 mg, Vet & 30 mg Vit $B_1$ 16 mg, Vit $B_2$ 50 mg Rantotheme and 100 mg, follic and 10 mg Niacin 300 mg, choline chloride 1000 mg $B_6$ 30 mg, $B_{12}$ 100 mg, biotin 250 mg and/iron, iodine, linc, mamganesese, copper Selemiun at 600, 5, 500, 600, 50, 1 mg respectively).

**Measurements:** Average weekly body weight development, gain and food consumption were recorded. Weekly food conversion efficiency was calculated according to Lambert et al. (1936). Blood samples were collected weekly and serum samples were kept for HI antibody titres estimation.

**NewCastle disease vaccination program:** Chicks of groups 1 to 4 were vaccinated against ND by ocular rout at age of 7 days using Hitchner $B_1$ strain vaccine (Ceva Lab's Inc., Overlard Park, KS 66212, List No. 5050-5041, Serial No. 09-637) containing $10^{10}$ $EID_{50}$/ml. Revaccination was done by La Sota strain vaccine (Ceva Lab's Inc., Overlard Park, KS 66212, List No. 5295, Serial No. 04-553). Containing $10^{10}$ $EID_{50}$/ml, in drinking water at age of 21 days.

**Determination of virus infectivity:** Titration of living virus before use was carried out according to Reed and Muench (1938).

**Haemagglutination inhibition (HI) test:** Was carried out according to the technique described by Takatsy (1956).

**Challenge NewCastle disease virus:** A velogenic strain of ND virus identified by Sheble and Reda (1977) was used after titration.

**Challenge test:** Birds of groups 1 to 5 were challeged against velogenic ND virus by intramuscular inoculation with 0.2 ml per bird containing $10^6$ $EID_{50}$ at age of 42 days.

## RESULTS AND DISCUSSION

The effect of vitamin E and selenium on the immune response of chicks against living ND vaccines as well as body weight development and food conversion efficiency were studied.

The results illustrated in **Table (2)** revealed that the HI antibody titres against ND virus before challenge at 42 days of age in group 1, which received 300 mg/kg vitamin E, was 4.67 and in group 2, which received 300 mg vitamin E and 0.25 mg selenium/kg diet, was 4.80. The HI titres in groups 1 and 2 were

Table.(2):Results of haemagglutinating inhibiting (HI) antibody titres and challenge test against velogenic NewCastle disease (ND) virus.

| Age in days | Group No. | No.of titrated serum samples. | HI antibody titres against ND virus expressed in TRN. 0 | 2 | 3 | 4 | 5 | 6 | 7 | Geometric mean of HI titres | Protection percentage |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | B.T$ | 15 | 2 | 7 | 5 | 1 | - | - | - | 2.20 | nd° |
| 7 | | 15 | 4 | 8 | 3 | - | - | - | - | 1.66 | |
| 14 | | 15 | 2 | 7 | 4 | 2 | - | - | - | 2.26 | |
| 21 | | 15 | - | 6 | 7 | 2 | - | - | - | 2.73 | |
| 28 | (1) | 15 | - | 4 | 7 | 3 | 1 | - | - | 3.07 | 84.00 |
| 35 | | 15 | - | 3 | 5 | 4 | 2 | 1 | - | 3.53 | |
| 42 | | 15 | - | - | 1 | 7 | 4 | 2 | 1 | 4.67[a] | |
| 14 | | 15 | 3 | 5 | 5 | 2 | - | - | - | 2.20 | |
| 21 | | 15 | - | 3 | 6 | 6 | - | - | - | 3.20 | |
| 28 | (2) | 15 | - | 2 | 8 | 3 | 2 | - | - | 3.33 | 92.00 |
| 35 | | 15 | - | 1 | 6 | 5 | 3 | - | - | 3.67 | |
| 42 | | 15 | - | - | 2 | 4 | 5 | 3 | 1 | 4.80[a] | |
| 14 | | 15 | 2 | 8 | 4 | 1 | - | - | - | 2.13 | |
| 21 | | 15 | - | 7 | 6 | 2 | - | - | - | 2.67 | |
| 28 | (3) | 15 | - | 4 | 6 | 3 | 2 | - | - | 3.20 | 80.00 |
| 35 | | 15 | - | 2 | 5 | 6 | 2 | - | - | 3.53 | |
| 42 | | 15 | - | 1 | 3 | 5 | 5 | 1 | - | 4.13[b] | |
| 14 | | 15 | 2 | 6 | 5 | 2 | - | - | - | 2.33 | |
| 21 | | 15 | 1 | 5 | 7 | 2 | - | - | - | 2.60 | |
| 28 | (4) | 15 | - | 4 | 5 | 5 | 1 | - | - | 3.20 | 76.00 |
| 35 | | 15 | - | 3 | 5 | 4 | 3 | - | - | 3.87 | |
| 42 | | 15 | - | - | 6 | 5 | 2 | 2 | - | 4.00[b] | |
| 14 | | 15 | 0 | 5 | 2 | - | - | - | - | 1.86 | |
| 21 | | 15 | 9 | 5 | 1 | - | - | - | - | 0.87 | |
| 28 | (5) | 15 | 12 | 3 | - | - | - | - | - | 0.40 | 00.00 |
| 35 | | 15 | 15 | - | - | - | - | - | - | 0.00 | |
| 42 | | 15 | 15 | - | - | - | - | - | - | 0.00[c] | |

°nd= not done.

$B.T= before treatment.

-Values with different superscriptes at the same column are significantly different at ($p<0.05$).

Group(1):supplemented with vit.E(300 mg/kg diet)and vaccinated.

Group(2):supplemented with vit.E(300 mg/kg)and selenium(0.25 mg/kg. and vaccinated.

Group(3):supplemented with selenium(0.25 mg/kg diet)and vaccinated

Group(4):unsupplemented vaccinated control.

Group(5):unsupplemented nonvaccinated negative control.

significantly ($P < 0.05$) higher than the titres of group 3, which received 0.25 mg selenium/kg diet, and the non-treated group 4. The previous results indicated that vitamin E enhanced HI antibody production. The enhancement effect of vitamin E in higher doses (3-6 times) over the currently used requirements on HI antibody production against ND virus was also observed by Law (1976); Franchini et al. (1983) and Farouk (1988). The mechanism by which vitamin E enhanced the production of HI antibodies could be explained on the suggestion of Tengerdy and Nockels (1973) who stated that vitamin E may act directly on antibody biosynthesis by an instantaneous regulation of protein biosynthesis, probably conected to its antioxidant regulatory role. On the other hand, vitamin E may play an indirect role in immuno-enhancement as antioxidant when it protects metabolic regulators such as ubiquinones or vitamin A from oxidation, or when it regulates the biosynthesis of prostaglandins through preventing the oxidation of arachidonic acid, (Tengerdy et al., 1981).

The protection percentage against challege with velogenic ND virus in **Table (2)** showed that group 2 recorded the highest protection rate (92%) follwoed by group 1(84%) and group 3 (80%), while group 4 recorded the lowest percentage (76%). The obtained result indicated that vitamin E and selenium supplementation together induced higher protection rate than the addition of each one alone compared with the vaccinated non-treated group (4). The synergestic action of vitamin E and selenium on improving immuno-response of chicks may be due to the association of such two elements with membrane fluidity of lymphoid cells (Sheffy and Schultz, (1979), or due to their effect on increasing phagocytic activity in several species (Tengerdy and Brown, 1977; Boyne and Arthur, 1979 and Likoff et al., 1981). Concering the role of vitamin E supplementation in inducing the optimal function of immune response and other host defense mechanism, when used in high levels, Tanaka et al. (1979) found that

Table(3): Average weekly body weight development (gms) and food conversion efficiency (FCE) during 6-weeks experimental period before challenge.

| Group No. | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Age in weeks. | Body weight | FCE | Body weight | FCE | Body weight | FCE | Body weight | FCE | Body weight | FCE |
| 1 | $65.7 \pm 8.4^{a}$ | 3.3 | $69.8 \pm 7.5^{a}$ | 3.5 | $70.4 \pm 8.2^{a}$ | 3.6 | $66.4 \pm 11.4^{a}$ | 3.7 | $64.5 \pm 10.3^{a}$ | 3.84 |
| 2 | $130.4 \pm 23.4^{a}$ | 3.6 | $129.2 \pm 21.2^{a}$ | 3.1 | $131.4 \pm 25.0^{a}$ | 3.4 | $129.3 \pm 19.4^{a}$ | 3.9 | $128.3 \pm 20.1^{a}$ | 3.95 |
| 3 | $178.2 \pm 35.6^{a}$ | 4.8 | $184.6 \pm 32.9^{a}$ | 3.9 | $170.4 \pm 28.6^{b}$ | 4.1 | $160.2 \pm 29.5^{c}$ | 4.0 | $165.4 \pm 33.0^{b}$ | 4.30 |
| 4 | $235.6 \pm 65.2^{b}$ | 4.4 | $245.8 \pm 59.3^{a}$ | 3.9 | $214.4 \pm 54.3^{c}$ | 4.7 | $220.6 \pm 49.6^{c}$ | 4.7 | $218.6 \pm 48.7^{c}$ | 4.85 |
| 5 | $340.7 \pm 45.4^{b}$ | 4.9 | $360.6 \pm 70.4^{a}$ | 4.0 | $336.6 \pm 87.6^{b}$ | 4.9 | $315.1 \pm 76.1^{c}$ | 4.9 | $300.6 \pm 79.1^{c}$ | 4.70 |
| 6 | $480.6 \pm 67.1^{a}$ | 5.0 | $487.0 \pm 73.9^{a}$ | 4.9 | $476.6 \pm 77.5^{b}$ | 5.3 | $469.6 \pm 80.4^{b}$ | 5.4 | $478.9 \pm 69.3^{b}$ | 5.50 |

($\pm$)= Standard deviation

-Statistical analysis (Analysis of variance) was done according to Senedecor(1956).

- Values with different superscriptes in the same raw were significantly differed at ($p<0.05$).

vitamin E promotes the prolifration of immunocopetent, antigen stimualted B lymphocytes, probably through an enhaced cooperation with T helper cells. Moreover , Colnago et al. (1984) explained that selenium (0.25 mg/kg diet) protects the leucocytes from self destruction during the phagocytic activity, therefore, the leucocytes retained at the site of infection protecting the host against the pathogens.

Regarding body weight development and feed conversion efficiency **Table (3)**, there was no significant difference between groups due to dietary treatment until the third week of age. Starting from the third week, the group of chicks (2) which received vitamin E and selenium at the rate of 300 and 0.25 mg/kg diet, acheived the significant ($P < 0.05$) highest body weight and best food conversion efficiency followed by group 1 (300 mg/kg vitamin E) compared with the other treated groups, and this result sustained to the end of the experiment. So, it could be noticed that vitamin E at rate of 300 mg/kg diet may improve body weight when given in sole as reported by Heizerling et al. (1974); Colnago et al. (1984) Zaki et al. (1989),but when it was supplemented with selenium (0.25 mg/kg), the synergestic improvement for body weight became more prominant. Such effect could be explained by the suggestion of Tengerdy and Nockels (1973), who obserbed a direct action of vitamin E supplementation at higher Ievels above the established requirements on protein biosynthesis.

Therefore, it is to be concluded that dietary supplementation of vitamin E (300 mg/kg) and selenium (0.25 mg/kg) before vaccination against ND with living vaccines enhanced the immune response of chicks as measured by HI antibody titres, protection against challenge with velogenic ND firus cohich was reflected on the body weight and feed conversion efficiency.

## SUMMARY

The immune response of chicks vaccianted with living Newcastle disease vaccines was significantly improved by vitamin E and selenium supplemented in diet 14 days before vaccination, at a ratio of 300 mg/kg and 0.25 ppm respectively, as measured by haemagglutination inhibition (HI) antibody titres and protection rate against challenge with velogenic Newcastle disease virus. Supplementation of vitamin E resulted in significant ($P < 0.05$) increase of the final body weight and reduction of feed convertion efficiency.

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