Department of Virology,
Faculty of Veterinary Medicine, Beni-Suef University.

INTERACTION BETWEEN VITAMIN E AND "CLONE 30" NEWCASTLE DISEASE VIRUS VACCINE

(With 4 Tables)

By

A.S. HUSSEIN

(Received at 11/6/2006)

تفاعل بين فيتامين هـ ولقاح مرض النيوكاسل (عترة كلون ٣٠)

أحمد سعد حسين

أجريت هذه الدراسة لمعرفة تأثير فيتامين هـ على لقاح مرض النيوكاسل (عترة كلون٣٠) ومقارنته بحقن الفيروس فقط في البيض المخصب، وقد كانت النتيجة الملحوظة أن انخفضت القوة العيارية للفيروس المختلط بفيتامين هـ بمقارنته بالقوة العيارية له فقط ، وتم تجميع السائل الجنيني للبيض بعد حقنه لاستخدامه في التجربة الثانية. في التجربة الثانية استخدم عدد ١٢٠ كتكوت عمر يوم وأجري التحصين عند عمر خمس أسابيع بعد تقسيم الكتاكيت الى ثلاث مجموعات متساوية ، المجموعة الأولي حصنت بلقاح كلون٣٠ فقط ، والثانية حقنت بلقاح كلون ٣٠ المخلوط مع فيتامين هـ داخل البيض، أما الثالثة فقد تركت بدون تحصين، وأجري تعريض الدجاج للعدوى بعد ٢١ يوم من الحقن لفيروس النيوكاسل المعدي، وقد كانت النتائج ملحوظة عند قياس المناعة حيث كانت أعلى نسبياً في المجموعة الثانية وكانت نسبة صد العدوي (الحماية) ٩٠%.

SUMMARY

Two experiments were conducted to determine the effect of addition of vitamin E on the infectivity titer of NDV vaccine (Clone 30), humoral immunity and protection against challenge in broiler chickens. The work done in this study has given a good evidence on the role of vitamin E when used during vaccination against NDV. In experiment one, the results given showed that there was a slight decrease in the titer of vaccinal strain of NDV (Clone 30) when inoculated with different concentrations of vitamin E. on the other hand, results of experiment two revealed that vitamin E plays a complementary role in the immune response (humoral immunity) of broiler chickens.

Key words: *Newcastle disease, vaccine, vitamin E, Clone 30*

INTRODUCTION

Newcastle disease (ND) constitutes a problem of major importance which is continuously threating the poultry industry all over the world. For control of the disease, vaccination is the only mean. The use of attenuated or lentogenic living virus vaccines is the method of choice particularly for mass vaccination.

Many studies on the biological activities of vitamin E have been carried out since it was first discovered by Evans and Bishop, 1923 and devoted a special attention to the influence of vitamin E on the immune system (Bartov & Frigg, 1992; Friedman *et al.*, 1998 and Kidd *et al.*, 2001). The stimulation of the immune response by vitamin E was greater when it was given in the first weeks of life, during the early development of the immune system (Franchini *et al.*, 1986). Also, several researches had indicated that relatively high concentrations of vitamin E added to the feed enhance the humoral immune response or disease resistance in poultry (Tengerdy *et al.*, 1973; Heinzerling *et al.*, 1974 ; Tengerdy *et al.*, 1981; Hag *et al.*, 1996 and Boa-Amponsem *et al.*, 2000). Gore and Qureshi, 1997, showed that vitamin E supplementation in ovo results in enhanced antibody and macrophage response in the chickens.

For all these reasons, several studies were conducted to assess a possible interaction between vitamin E and NDV vaccines. The objective of this study was to evaluate the effect of vitamin E on humoral immune response of broilers vaccinated with Clone 30 vaccine.

MATERIALS and METHODS

Chicks:

One-day old chicks (local breed) was purchased from commercial source at Beni-Suef Governorate. These chicks were housed under good hygienic measures in previously cleaned and disinfected rooms and fed on a balanced commercial ration. Some chicks were randomly selected and tested to assess the maternally derived HA NDV antibodies by using the HI test which repeated periodically till it showed that all chicks had become nearly susceptible to the NDV (five weeks old).

Embryonated chicken eggs (ECE):

Specific pathogen free (SPF) embryonated chicken eggs were obtained from Ministry of Agriculture & Cultivation of Lands,

production of SPF Embryonated Eggs Project, Kom Oshim, El-Fayoum Governorate incubated and used at 9-10 days old for:

1-Estimation of EID_{50} of the used virus.

2-Examination of the effect of vitamin E on the infectivity titer of the used virus

Newcastle disease virus (NDV):

A field strain of NDV (local field isolate) was isolated from a sever outbreak of ND. The strain was identified and characterized as VVNDV and was used for challenge of NDV-vaccinated and control chickens (El-Kady and Madbouly, 1997).

Live Newcastle disease virus vaccine:

Nobilis vaccine Clone 30 was derived by cloning the NDV LaSota Nobilis in chicken kidney cells. It was obtained from Intervet International . the EID_{50} was $10^{8.83}$/ml.

Vitamin E: from Rovigypt (Vitamin Rovimix ESOSD) vitamin E content was minimum 50% of alpha–tocopherol acetate

Buffers-:

physiological buffer saline: It was prepared as 0.85% NaCl according to Hudson & Hay, (1980) and used in haemagglutination inhibition test.

phosphate buffer saline: It was prepared according to Hudson & Hay (1980), sterilized by autoclaving and used diluent when ECE were used for virus titration.

Experimental designs:

Experiment (1): Effect of vitamin E on the infectivity titer of NDV strain (Clone 30) inoculated and propagated into ECE:

1- Infectivity titration of NDV vaccine (Clone 30) in embryonated chicken eggs:

Estimation of 50% end point was carried out by the method of Reed & Muench, (1938).

2- Preparation of vitamin E /NDV vaccine: three gram of vitamin E were dissolved in two milliliters of sterile saline and divided into four equal volumes (750mg vitamin E/1/2 ml). Four dilutions of the virus from 10-6 to 10-9 were used and mixed with vitamin E, 0.5ml from each dilution of the virus was added to 0.5ml of vitamin E containing 750mg. The four dilutions of the virus mixed with vitamin E were incubated at 37°C for an hour. Each dilution was inoculated into five eggs, each egg was inoculated with 0.2ml containing 150mg vitamin E. The inoculated eggs were incubated at 37°C for six days (deaths within 24 hours >>> non specific death). The EID_{50} were calculated using HA test and the result was calculated according to Reed and Muench. The

harvested allantoic and amniotic fluids from the positive HA eggs were used in experiment two to detect the potentiality of the vaccine. Sterility test was performed to ensure the sterility of the prepared vaccine.

Experiment (2): Effect of vaccination with Clone 30 alone or in combination with vitamin E on the immune response of broilers: a total of (120) 5-weeks old chicks were divided into three equal groups (40 chickens/group) and were treated as follows:

Group (1)>>> vaccinated with Clone 30 vaccine alone.

Group (2)>>> vaccinated with allantoic and amniotic fluids collected from experiment one.

Group (3)>>> kept as control non-vaccinated.

Each chick was inoculated intra muscularly with a 0.5ml vaccinal dose.

Table 1: groups of experiment 2:

Group	No. of birds	Treatment
1	40	Vaccinated by Clone 30 vaccine (EID50=$10^{8.83}$/ml)
2	40	Vaccinated by allantoic and amniotic fluids collected from experiment 1 vitamin E/NDV (EID_{50}= $10^{8.83}$/ml, vitamin E (150mg/egg).
3	40	Control non vaccinated.

Haemagglutination test (HA):

It was carried out as the standard method described by (Anon, 1971).

Serology:

All chicks were bled at one week intervals post vaccination over three weeks for individual HI test which was carried out according to the procedures adopted by Beard & Wilkes, (1973).

Challenge test:

The vaccinated and control non vaccinated birds from each group were subjected to challenge test 21 days post vaccination by I/M inoculation of $2X10^5$ ELD_{50} of VVNDV. The birds were observed for 15 days post inoculation and deaths within this period were subjected to post mortem examination.

RESULTS

Table 2: Efficacy of vitamin E on the infectivity titer of 10 day old ECE using Clone 30 vaccine with different doses of vitamin E:

Original EID_{50}/ml of Clone 30 vaccine	Vitamin E concentration	EID_{50}/ml of virus after mixing with vitamin E	Log difference
$10^{8.83}$	150mg/egg	$10^{7.69}$	1.14
	75mg/egg	$10^{8.3}$	0.53
	37.5	$10^{7.7}$	1.13

The quality control of the prepared vaccine: The prepared vaccine are sterile (not contained any bacterial, fungal, and/or mycoplasmal contaminants when cultured on the corresponding media used for their cultivation). They also are safe when inoculated in either SPF-ECE or in susceptible chicks.

Table 3: Mean log2 HI antibody titers of chickens vaccinated with Clone 30 alone and combined with vitamin E weekly post-vaccination:

Chicken group	Mean log_2 HI antibody titers weekly post-vaccination		
	1st week	2nd week	3rd week
1	2.2	3.5	5.4
2	2.9	3.63	6.2
3 (Control non-vaccinated)	0	0	0

Table 4: Protection percentages of the chickens challenged at 21 days post vaccination:

Chicken group	No. of birds	No. of survived	Protection percentages
1	40	33	82.5%
2	40	36	90%
3	40	0	0%

DISCUSSION

Vitamin E or alpha tocopherol acetate is fat soluble vitamin that is essential for the proper nutrition of chicken and turkeys. It is associated with a variety of functions affecting the reproductive, nervous, circularity, muscular and haemotopiotic system (Scott 1980, puthpongsiriporn *et al.*, 2001 and Muir *et al.*, 2002). Vitamin E has been shown to directly influence the immune system of birds under several experimental models (Erf *et al.*, 1998; Friedman *et al.*, 1998 and Kidd *et al.*, 2001).

In this work, two experiments were conducted to assess a possible interaction between vitamin E and NDV vaccines. The first study was conducted to examine the effect of addition of vitamin E on the infectivity titer of NDV (Clone 30). The second was to verify the effect of vaccination with Clone 30 vaccine mixed with vitamin E on the immune response of the broilers.

In experiment (1), The results given showed the effect of different doses of vitamin E on the EID_{50} of 10 day old ECEs. It was noticed that the embryo remained alive during the observation period (6 days) in case of eggs inoculated by NDV with vitamin E compared to those inoculated with NDV vaccine alone. Results in Table (1) showed that there was slight decrease in the titer of the NDV vaccine when inoculated with different concentrations of vitamin E. The log difference between NDV vaccine alone and in combination with vitamin E ranged between (0.53 and 1.14). There was no marked differences in titer between the different doses of vitamin E.

Unfortunately there was no available data regarding the role of vitamin E on the infectivity titer of NDV inoculated and propagated in ECE. The decreased in the titer may be due to the effect of vitamin E on

the fat content of the NDV, as vitamin E is a fat soluble vitamin. This process can lead to some changes in the multiplication cycle of the virus.

Experiment (2) was then planned to study the effect of using a combination of vitamin E and Clone 30 vaccine on the immune response of the susceptible chickens. In this experiment, three groups of one-day old chicks were reared in isolation without vaccination till five-weeks old of age, then each group was subjected to different treatments; chicks of group (1) vaccinated with Clone 30 vaccine alone, group (2) vaccinated with combined NDV vaccine/vitamin E which prepared in experiment (1). Chickens of group three used as control non-vaccinated group (Table 1).

The mean $\log_2$ HI antibody titer of the vaccinated chickens began to increase gradually from the first week post vaccination till the third week post vaccination. (illustrated in table 3). From this table it is clear that chickens vaccinated with allantoic and amniotic fluids contained Clone 30 and vitamin E had high HI antibody titer (2.9, 3.63 and 6.2)comparing with that vaccinated with Clone 30 alone (2.2, 3.5 and 5.4) at 1^{st}, 2^{nd} and 3^{rd} week post vaccination respectively. However, the relatively higher HI antibody titer induced in the chickens of group two may be due to the inoculation of liquid vaccine containing vitamin E which gave high multiplicity multiplication more than freeze dried vaccine. At the same time, group one was vaccinated with freeze dried vaccine and the virus dose not always multiply to high titer on the subsequent first passage (Palya *et al.*, 1991).

Concerning the evaluation of the immune response by challenge test, data presented in Table (4) revealed that the protection percentages of the challenged chickens were 82.5% and 90% in groups 1 and 2 respectively.

There was a close correlation between the HI antibody titer of different groups and protection levels. This result is supported by the fact that protection against NDV challenge is mainly dependent upon the presence of sufficient amounts of antibody titers (Box and Furminger 1975 and Bennejean *et al.*, 1978).

Finally, from the fore mentioned results, one arrive at the conclusion that vitamin E used with live vaccines has a benefit effect regarding the humoral immune response of broiler chickens. Further research is needed to test the efficacy of vitamin E on actual viral challenge.

REFERENCES

Anon, (1971): Methods for examining poultry biologics and for identifying & quantifying avian pathogens. National Academy of Science Washington, D.C.

Bartov, I. and Frigg, M. (1992): Effect of high concentration of dietary vitamin E during various age periods on performance, plasma vitamin E amd meat stability of broiler chicks at 7 weeks of age. Br. Poult. Sc., 33: 393-402.

Beard and Wilkes, (1973): A simple and rapid microtest procedure for determining Newcastle haemagglutination-inhibition antibody titers. Proce. 77th Ann. Mtg. USA An. Health Assoc., 596-600

Bennejean, G.; Guittel, W. and Picault, J.P. (1978): vaccination of one-day old chicks against ND using inactivated oil adjuvant vaccine and/or live vaccine. Av. Path. 7:15-27. Avian Dis. 1990 Jan-Mar; 34(1): 154-62.

Boa-Amponsem, K.; Price, S.H.; Picard, M.; Geraert, P.A. and Siegal, P.B. (2000): Vitamin E and immune responses of broiler pureline chickens. Poult. Sci., 79, 466-470.

Box, P.G. and Furminger, I.G.S. (1975): ND antibody levels in chickens after vaccination with oil emulsion adjuvant killed vaccine. Vet. Rec., 96: 108-111.

*El-Kady, M.F. and Madbouly, H.M. (1997):*Antigenic variation among some local Newcastle disease virus isolates. Alex. J. Vet. Sci., Vol. 13, No. 7, 913.

Erf, G.F.; Bottje, W.G.; Bersi, T.K.; Headrick, M.D. and Fritts, C.A. (1998): Effects of dietary vitamin E on immune system in broilers, Altered proportions of CD4 T cells in the thymus and spleen. Poult. Sci.,77: 529-537.

Evans, H. and Bishop, K. (1923): On the existence of hitherto unrecognized factor essential for reproduction. Science, 56:650-651.

Franchini, A.S. and Meluzz, (1986): The influence of high doses of vitamin E on immune response of chicks to inactivated oil adjuvant vaccine. Clin. Vet., 109:117-127.

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

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.

Hag, A.; Baily, C.A. and Chinnah, A. (1996): Effect of b-carotene, canthaxanthin, lutein, and vitamin Eon neonatal immunity of chicks when supplemented in the broiler breeder diets. Poult. Sci., 75: 1092-1097.

Heinzerling, R.H.; Nockels, C.F.; Quarles, C.L. and Tengerdy, R.P. (1974): Protection of chicks against E.coli infection by dietary supplementation with vitamin E. Proc. Soc. Exp. Biol. Med. 146: 279-283.

Hudson, L. and Hay, D.F. (1980): Practical immunology. 2nd ed., Blackwell Sci. Pub., Oxford, London, Boston pp.122-152.

Kidd, M.T.; Peebles, E.D.; Whitmarsh, S.K.; Yeatman, J.B. and Wideman, R.F. (2001): Growth and immunity of broiler chicks as affected by dietary arginine. Poult. Sci., 80: 1535-1542.

Muir, W.I.; Husband, A.J. and Bryden, W.L. (2002): Dietary supplementation with vitamin E modulates avian intestinal immunity. Br. J. Nutr., 87: 579-585.

Palya, V.; Horváth, T. and Lomniczi, B. (1991): Examination of field virus excretion in chickens vaccinated against Newcastle disease. 2. Excretion of two recent NDV strains from chickens vaccinated twice by different routes. Poult. Sci., 80: 1150-1162.

Puthpongsiriporn, U.; Scheideler, S.E. and Sell, J.L. (2001): Effects of vitamin E and C supplementation on performance, in vitro lymphocyte proliferation, and antioxidant status of laying hens during heat stress. Poult. Sci., 80: 1190-2000.

Reed, L.J. and Muench, H. (1938): Simple method of estimating fifty percent end points. Am. J. Hyg., 27: 493-497.

Scott, M.L. (1980): Advances in our understanding of vitamin E. Fed.Proc., 39: 2736-2739.

Tengerdy, R.P.; Heinzerling, R.H.; Brown, G.L. and Mathias, H.H. (1973): Enhancement of the humoral immune response by vitamin E. Int. Arch. Allergy 44: 221-232.

Tengerdy, R.P.; Mathias, M.M. and Nockels, C.F. (1981): Vitamin E. immunity and disease resistance. Pages 27-42 in: Diet and resistance to disease. M. Phillips and A. Baetz, ed. Plenum Press, New York, NY.