

## EFFECT OF VITAMIN-MINERAL PREMIX ON IMMUNE RESPONSE OF BABY CHICK RANIKHET DISEASE VACCINE IN CHICKS

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### ABSTRACT

A total of 150 (75 broiler and 75 layer) apparently healthy chicks were used to investigate the effect of vitamin-mineral premix on immune responses of Newcastle disease (ND) vaccine (Baby Chick Ranikhet Disease Vaccine, F-strain) during the period from January to March 2003. The chicks were divided into six groups namely group A, B and C for broiler and group D, E and F for layer birds consisting of 25 birds in each group. Chicks of group A, C, D and F were supplied with vitamin-mineral premix containing feed and chicks of group B and E were supplied with vitamin-mineral deficient feed. The chicks of group A, B, D and E were primarily vaccinated with 0.1 ml of BCRDV of  $10^{6.5}$  EID<sub>50</sub> / 0.1 ml per bird through intraocular route at the age of day 3 and were revaccinated with the same vaccine at the age of day 17. Birds of group C and F were kept as unvaccinated control. Chickens of all the groups were challenged with the virulent isolate of NDV @ 0.5 ml/bird IM ( $10^{4.5}$  EID<sub>50</sub>/0.1 ml) after two weeks of secondary vaccination. Titres were determined before and after vaccination by hemagglutination test (HI) test and serum neutralization test (SNT) was performed to measure the potency of immune serum. The HI titres of birds of groups A, B, D and E were 128, 16, 128 and 128 respectively after primary vaccination and 256, 128, 256 and 256 respectively after secondary vaccination. From the study it was found that among the experimental layer birds, no difference was noticed on the immune response to BCRDV. On the other hand, among the experimental broiler birds, birds of group A revealed higher HI titre compared to the birds of group B. Results of serum neutralization test indicated that the sera of birds possessing HI titre  $\geq 128$  demonstrated a good level of virus neutralizing activity of  $\geq 7.60$ . The birds possessing HI titre of  $128 \pm 0.00$  did not show any clinical signs of ND following challenge and the survivability was recorded as 100%. It may be concluded that supplementation of vitamin-mineral premix to the diet induces better immune response following vaccination with BCRDV in broiler chickens compared to the layer chickens.

**Key words:** Effect, vitamin-mineral premix, immune response, Ranikhet disease, chicks

### INTRODUCTION

Newcastle disease (ND) is identified as a deadly viral disease of poultry all over the world. Among the domestic birds, chickens of different ages and breeds are considered to be most susceptible birds. ND may cause 100% mortality in young chicks and 80-90% mortality in adult birds (Brandly, 1950). The poultry farmers of both urban and rural areas are very much afraid of sudden attack of their birds with ND virus (NDV) of either velogenic or mesogenic strains in Bangladesh. To reduce the mortality rate due to ND proper vaccination with an effective vaccine strain has no alternative. Vaccines produced from mesogenic and lentogenic strains are successfully used in Bangladesh. Vitamin-mineral premix is the combination of vitamins and minerals, which is added to prepare balanced poultry diet, is now a usual practice. Certain trace minerals like selenium, copper, zinc, cobalt and iron have importance in immune system as they have been seen to alter various components of the immune system (Suttle and Jones, 1989). Poultry under intensive production system are particularly susceptible to vitamin-mineral deficiencies. Vitamin-mineral premix provides margin of safety and are adequate under stress conditions to which chickens may be exposed to various infectious agents (Scott *et al.*, 1982). Any remarkable research relation to the influence of vitamin-mineral premix on the immune phenomenon of chicken with ND vaccination has so far not been conducted in Bangladesh. Therefore, the present study was undertaken to investigate the effect of vitamin-mineral premix on the immune responses following vaccination with live ND vaccine (Baby Chick Ranikhet Disease Vaccine) in chicks.

### MATERIALS AND METHODS

The effect of vitamin-mineral premix on the immune response of ND vaccine was determined by using 150 (75 broiler and 75 layer) apparently healthy chicks, which was conducted in the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh from January to March 2003.

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### Birds, feed, vaccine and virus

A total of 75, day-old chicks of White Leghorn layer chicks without ND vaccination were purchased from Goalundo Hatchery and 75 Star bro broiler chicks without ND vaccination were purchased from the Begum Rokeya Poultry Farm, Mymensingh. All the layer and broiler chicks were reared separately in a well ventilated poultry house of the Department of Microbiology and Hygiene, BAU, Mymensingh with *ad libitum* feed and water.

Vitamin-mineral premix and concentrates (Provita<sup>®</sup>, 250 gm/100 kg feed, USA) and vitamin A capsules (Retinol Forte<sup>®</sup>, 50000 IU/Cap, Drug International) were used throughout the experiment. Lyophilized Baby Chick Ranikhet Disease Vaccine (BCRDV- ND vaccine with F-strain and containing a titre  $10^{6.5}$  EID<sub>50</sub>/0.1 ml) produced by the Livestock Research Institute (LRI), Mohakhali, Dhaka was used for the study. A virulent local isolate of NDV was obtained from the laboratory repository of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh and was used as stock virus for the determination of serum antibody titre by hemagglutination inhibition and serum neutralization test.

### Vaccination of chicks

The layer chicks were divided into three equal groups namely group A, B and C. Similarly broiler chicks were divided into groups D, E and F. Chicks of group A, C, D and F were supplied with vitamin-mineral premix containing feed and chicks of group B and E were supplied with feed without vitamin-mineral premix. Chicks of group A, B, D and E were primarily vaccinated with one drop of live ND vaccine (BCRDV) in one eye of each bird primarily at the age of day 3 and boosted at 17 days old. The chicks of group C and F were kept as unvaccinated control. Blood of chicks of all the groups were collected for sera to determine antibody titre before and after vaccination.

### Challenge Test

Chickens of all the groups were challenged with the virulent isolate of NDV @ 0.5 ml/bird contained virus of  $10^{4.5}$  EID<sub>50</sub>/0.1 ml through IM route after two weeks of secondary vaccination.

### Hemagglutination inhibition test

The Hemagglutination inhibition (HI) test was conducted following the  $\beta$ -method (a constant virus of 4HA unit was used in each well of a serially diluted serum containing well of the microplates) as described by Stephen *et al.* (1975) to determine the HI titre of the test sera samples collected from the vaccinated and unvaccinated birds used in this study.

### Serum neutralization test

The serum neutralization test (SNT) was conducted to measure the potency of serum of the vaccinated groups of birds by determining the degree of virus neutralizing capacity of the immune serum. To determine the virus neutralizing capacity, the virulent NDV of a definite dilution ( $10^{-1} - 10^{-10}$ ) and the immune sera of different vaccinated groups of birds having hemagglutination inhibition titre of <4, 8, 16, 128 and 256 were used. The test was carried out by inoculation of serum-virus mixture in 10-day-old chicken embryo through allantoic cavity route and observed for changes within 5 days. The EID<sub>50</sub> of the virus and the serum-virus mixture were calculated separately according to the method described by Reed and Muench (1938). The neutralizing activity of serum was calculated as the differences observed between the antilogarithm of the log<sub>10</sub>EID<sub>50</sub> of virus and log<sub>10</sub>EID<sub>50</sub> of serum virus mixture.

## RESULTS AND DISCUSSION

The HI titres were found  $128 \pm 0.00$  and  $16 \pm 0.00$  in the birds of group A and B and  $128 \pm 0.00$  and  $128 \pm 0.00$  in the birds of group D and E respectively after two weeks of primary vaccination. Similarly two weeks after secondary vaccination the HI titres were  $256 \pm 0.00$ ,  $128 \pm 0.00$ ,  $256 \pm 0.00$  and  $256 \pm 0.00$  in the birds of group A, B, D and E respectively (Table 1). The sera of vaccinated groups of chicken having hemagglutination titre of 256, 128, 16, 8 and <4 were found to neutralize the EID<sub>50</sub> of virus  $10^{8.40}$ ,  $10^{7.60}$ ,  $10^{6.10}$ ,  $10^{5.22}$  and  $10^{4.80}$  respectively. The highest neutralizing activity of sera having HI titre was found  $10^{8.40}$  in relation to EID<sub>50</sub> of the virus (Table 2).

In this study it is found that no significant variation of HI titres in different groups of layer birds which were primarily vaccinated with live ND vaccine (BCRDV) through intraocular route at the age of day 3. This finding has a close relationship with the findings of Coskun *et al.* (1998) and Lin *et al.* (2002) who reported that vitamin A had no significant effect on the antibody titres in layer birds. The chicks of different layer groups were also re-vaccinated with the same live ND vaccine at the age of day 17 and found no significant variation in the titres also.

Table 1. Antibody titres with challenge results of broiler and layer chickens immunized with Baby Chick Ranikhet Disease Vaccine (BCRDV)

Breed of birds	Group of chicks	No. of birds	Pre-vaccination titre	HI titres two weeks after vaccination (Mean ± SE)		Challenge results	
				Primary	Secondary	No. survived	%
Broiler (Starbro)	A (premix containing feed and vaccinated)	25	8	128 ± 0.00	256 ± 0.00	25	100
	B (premix deficient feed and vaccinated)	25	8	16 ± 0.00	128 ± 0.00	25	100
	C (premix containing feed and unvaccinated control)	25	8	<4	<4	00	00
Layer (White leghorn)	D (premix containing feed and vaccinated)	25	<4	128 ± 0.00	256 ± 0.00	25	100
	E (premix deficient feed and vaccinated)	25	<4	128 ± 0.00	256 ± 0.00	25	100
	F (premix containing feed and unvaccinated control)	25	<4	<4	<4	00	00

Table 2. Neutralizing activities (Neutralization index) of serum samples collected from birds of different vaccinated groups

HI titres of serum	Virus dilution and serum mixture						EID <sub>50</sub> of serum virus mixture	Virus dilution					EID <sub>50</sub> of virus	NI of diluted serum	Serum dilution used	NI of undiluted serum	
	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>		10 <sup>-7</sup>	10 <sup>-7</sup>	10 <sup>-8</sup>	10 <sup>-9</sup>	10 <sup>-10</sup>					10 <sup>-11</sup>
<4			5/5	3/5	2/5	0/5	10 <sup>5.5</sup>	5/5	5/5	4/5	3/5	1/5	0/5	10 <sup>10.25</sup>	10 <sup>4.5</sup>	1:2	10 <sup>4.80</sup>
8		5/5	4/5	3/5	1/5	0/5	10 <sup>5.25</sup>	5/5	5/5	4/5	3/5	1/5	0/5	10 <sup>10.25</sup>	10 <sup>4.75</sup>	1:2	10 <sup>5.22</sup>
16		5/5	4/5	2/5	1/5	0/5	10 <sup>4.75</sup>	5/5	5/5	4/5	3/5	1/5	0/5	10 <sup>10.25</sup>	10 <sup>5.5</sup>	1:2	10 <sup>6.10</sup>
128		3/5	2/5	0/5	0/5	–	10 <sup>3.5</sup>	5/5	5/5	4/5	3/5	1/5	0/5	10 <sup>10.25</sup>	10 <sup>6.75</sup>	1:2	10 <sup>7.60</sup>
256	<sup>a</sup> 4/5 <sup>d</sup>	2/5	1/5	0/5	0/5	–	10 <sup>2.75</sup>	5/5	5/5	4/5	3/5	1/5	0/5	10 <sup>10.25</sup>	10 <sup>7.5</sup>	1:2	10 <sup>8.40</sup>

n = Number of embryos died, d = Number of embryos inoculated.

Among the vaccinated groups of broiler birds, the birds which were fed balanced diet with vitamin A and mineral premix showed better immune response after primary and secondary vaccination with BCRDV than the birds of the group deficient with vitamin and mineral in their normal ration. This finding has close relationship with the findings of Mazija *et al.* (1992), Sklan *et al.* (1994) and Davis and Sell (1989). The result of challenge test indicated that the birds possessing serum HI titre of 128 ± 0.00 did not show any clinical signs of ND in the challenge infection and the rate of survivability was recorded as 100% whereas all the birds of control groups showed characteristics signs of illness and died within 10 days of post challenge (Table 1). From the study, it may be concluded that the addition of Vitamin and mineral premix in normal diet helps in the induction of immune response in birds vaccinated with BCRDV and better immune response is observed in broiler birds compared to layer birds.

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