



Humoral Immunological Response in Broilers Vaccinated Against Newcastle Disease and Supplemented with Dietary Zinc and Vitamin E

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ABSTRACT

The humoral immune response of broilers fed diets supplemented with Zinc (Zn) (0, 40, and 400mg/kg) and vitamin E (0, 12, and 120mg/kg), separately or combined, was evaluated. A completely randomized design was used according to a 3x3 factorial arrangement, with nine treatments and four repetitions per treatment. Sexed birds (n=1440) were distributed in 36 boxes with 40 chicks each and reared until 41 days of age. Vaccination against Newcastle Disease (ND) was performed when birds were 14 days old. ELISA and HI tests were used to assess antibody levels before vaccination and at 28, 35 and 41 days of age. The results were analyzed using Proc Mixed from SAS. The interaction between the highest dietary levels of Zn and vitamin E resulted in higher antibody levels in ELISA at 14, 28, 35 and 41 days of age. Increasing levels of Zn and vitamin E supplemented separately in the diets provided greater levels of hemagglutinating antibodies in HI at 14, 28, 35 and 41 days old. The interaction between the highest levels of Zn and vitamin E resulted in greater antibody levels at 41 days of age.

INTRODUCTION

Immunological response might be influenced by several factors. Among them, the nutritional condition of the animal subjected to antigenic challenge has been widely studied, including the effects of zinc and vitamin E.

Vitamin E acts effectively on the immunological system either by influencing immune cell proliferation (Tengerdy & Brown, 1977), or by its antioxidant and protective activity on cell membrane integrity.

The National Research Council (NRC) (1994) suggests that vitamin E should be supplemented at 12mg/kg in broiler diets throughout rearing. However, higher levels have been used in some studies, as a means of improving immune response (Tengerdy & Nockels, 1973). On the other hand, vitamin E supplementation has actually improved the responses of the humoral immune system in comparison to the recommended standard levels (Tengerdy & Brown, 1977).

Zinc is an essential micro-mineral that is involved in several metabolic routes that are fundamental for growing and living (Maiorka & Macari, 2002). According to NRC (1994), the recommended dietary level is 40ppm for broilers. This mineral is essential for the immunological function and disease prevention, since it improves the responses to antigenic challenges under field conditions (Kidd *et al.*, 1996). Adequate Zn consumption is crucial to the development, maintenance and efficient functioning of the immunological system and the cells associated to it (Dardenne & Bach, 1993). Nevertheless, previous studies have not proven that zinc has influence on chick immunological response (Kidd *et al.*, 1992; Pimentel & Cook, 1988; Pimentel *et al.*, 1991).



Bettger *et al.* (1980) reported that some diseases associated to Zn deficiency may be prevented with higher levels of antioxidant in the diets. Besides, vitamin E was the most effective agent against Zn deficiency, evidencing a significant physiologic interaction between vitamin E and Zn supplementation in broiler diets.

Cellulites incidence decreased in broilers fed an association of Zn and vitamin E (Downs *et al.*, 2000). There was an additive effect between these nutrients and therefore it may be suggested that the interaction may improve broiler performance and humoral immunological response.

The aim of this study was to evaluate the influence of different levels of Zn and vitamin E supplementation both separately and combined on broiler humoral immunological response before and after vaccination against Newcastle Disease (ND).

MATERIAL AND METHODS

In this study, 1440 one-day-old-broiler Hybro chicks were housed in 36 pens with 40 chickens each (50% males and 50% females). A completely randomized design was used according to a 3x3 factorial arrangement, with nine treatments and four repetitions per treatment. Corn- and soybean meal-based diets were formulated according to nutritional levels commonly used in commercial poultry farms. There were three levels of zinc (0, 40 and 400mg/kg) in the form of chelated zinc, and three levels of vitamin E (0, 12 and 120mg/kg) in the form of DL-2-tocopherol-acetate. Therefore, nine diets were prepared with the following levels of zinc and vitamin E per kg of feed:

- 1) No zinc or vitamin E;
- 2) 40mg Zn;
- 3) 400mg Zn;
- 4) 12mg vitamin E;
- 5) 40mg Zn and 12mg vitamin E;
- 6) 400mg Zn and 12mg vitamin E;
- 7) 120mg vitamin E;
- 8) 40mg Zn and 120mg vitamin E; and
- 9) 400mg Zn and 120mg vitamin E. All birds were supplemented until 41 days of age.

At 14 days old, the chickens were vaccinated using the eyedrop technique against Newcastle disease, using a commercially available LaSota strain. Before vaccination, blood samples were randomly collected by heart puncture from five broilers per repetition. At the 21, 35 and 41 days of age, blood samples were randomly collected from the ulnar vein of ten fowls

from each repetition. Serum samples were separated and stored at -20°C for further analyses.

Newcastle antibody levels in serum samples were analyzed using hemmagglutination inhibition (HI) according to Cunningham (1971) and enzyme-linked immunosorbent assay (ELISA) using a commercial kit according to the instructions provided by the manufacturer (Idexx Laboratories). The antibody levels were expressed as Geometric Mean Titers (GMT) and statistically analyzed using SAS® (SAS Institute, 2000). The hypothesis of the analysis of variance was tested using the LAB ProcMixed procedure of SAS. A polynomial regression analysis was then performed at 5% of significance level.

RESULTS

The results of antibody levels in the sera of broilers (GMT) obtained in ELISA and HI are shown in Tables 1 and 2, respectively. In both tests, antibody levels in all samples were significantly affected ($p < 0.05$) by both Zn and vitamin E, and also by the combination between them.

Table 1 - Geometric Mean Titers (GMT) of antibodies against the Newcastle Disease Virus measured by ELISA in the serum of broilers at 14, 28, 35 and 41 days of age.

Vitamin E (mg/kg)	Zinc (mg/kg)	GMT (ELISA) Age (days)			
		14	28	35	41
0	0	196.75	1627.50	2205.25	2075.25
	40	215.25	1902.75	2398.50	2122.00
	400	279.75	2284.00	3120.50	2822.50
12	0	223.00	2172.00	3108.75	2789.00
	40	234.25	2217.00	3189.75	2804.75
	400	295.75	2301.25	3383.75	2890.25
120	0	298.00	2351.25	3449.75	3037.50
	40	311.00	2692.75	4220.25	3401.50
	400	316.00	2785.00	4320.00	3792.00
SEM		6.86	41.70	33.56	39.10
CV (%)		5.21	3.69	2.06	2.74

SEM = standard error of the mean, CV = coefficient of variation.

Table 2 - Geometric Mean Titers (GMT) of antibodies against the Newcastle Disease Virus assessed by HI in the serum of broilers at 14, 28, 35 and 41 days of age.

Vitamin E (mg/kg)	Zinc (mg/kg)	GMT (HI) Broilers age (days)			
		14	28	35	41
0	0	2.29	39.53	65.38	55.91
	40	2.46	41.66	68.85	60.78
	400	2.73	49.50	92.24	81.87
12	0	2.59	44.41	77.56	64.23
	40	2.63	49.77	90.61	80.40
	400	2.83	55.78	95.73	85.96
120	0	2.94	57.71	109.69	92.24
	40	3.04	67.53	121.57	100.61
	400	3.16	70.08	130.45	109.56
SEM		0.15	3.27	3.49	2.62
CV (%)		11.27	12.39	7.39	6.45

SEM = standard error of the mean, CV = coefficient of variation.



ELISA Test

At 14 days, there was a marked linear increase on mean antibody levels with increasing levels of Zn, at 0 and 12mg/kg of vitamin E, according to the following equations:

$$\text{GMT}=0.196x+201.79 \text{ (R}^2=0.98) \text{ and}$$

$$\text{GMT}=0.1775x+224.97 \text{ (R}^2=0.99), \text{ respectively, in}$$

which the independent variable is zinc level. However, mean antibody levels did not change considerably at 120mg/kg of vitamin E. A significant linear increase occurred on antibody levels due to increasing levels of vitamin E within all levels of Zn (0, 40 and 400mg/kg), according to the following equations:

$$\text{GMT}=0.7847x+204.72 \text{ (R}^2=0.97);$$

$$\text{GMT}=0.7621x+220.05 \text{ (R}^2=0.99), \text{ and}$$

$\text{GMT}=0.2568x+285.87 \text{ (R}^2=0.87)$, where X corresponds to the levels of vitamin E.

At 28 days, mean antibody levels showed a significant increase due to increasing levels of zinc within each vitamin E level. Antibody levels varied according to the following equations at 0, 12 and 120 mg/kg of vitamin E, respectively:

$$\text{GMT}=1.4109x+1731.1 \text{ (R}^2=0.89),$$

$$\text{GMT}=0.2879x+2187.9 \text{ (R}^2=0.93), \text{ and}$$

$\text{GMT}=0.7568x+2498.7 \text{ (R}^2=0.53)$, in which X correspond to the different Zn levels. For 0, 40 and 400mg/kg of Zn, there was a significant linear increase in the responses of antibody levels with the different levels of vitamin E based on the following equations:

$$\text{GMT}=4.3019x+1861 \text{ (R}^2=0.57),$$

$$\text{GMT}=5.7216x+2019.1 \text{ (R}^2=0.90), \text{ and}$$

$\text{GMT}=4.2953x+2267.8 \text{ (R}^2=0.99)$, respectively, in which X is the level of vitamin E.

At 35 days, there was a marked increase in antibody levels due to increasing amounts of Zn, within each vitamin E level. At 0, 12 and 120mg/kg of vitamin E, behavior is shown by the equations:

$$\text{GMT}=2.1763x+2255.6 \text{ (R}^2=0.99),$$

$$\text{GMT}=0.6287x+3135.2 \text{ (R}^2=0.96), \text{ and}$$

$\text{GMT}=1.4246x+3787.7 \text{ (R}^2=0.43)$, respectively, in which X represents Zn levels. At 0, 40 and 400mg/kg of Zn, there was a significant linear rise in antibody response at increasing levels of vitamin E (X) that may be expressed according to the following equations:

$$\text{GMT}=7.5172x+2590.5 \text{ (R}^2 = 0.60),$$

$$\text{GMT}=12.95x+2699.7 \text{ (R}^2 = 0.88), \text{ and}$$

$$\text{GMT}=9.4709x+3191.4 \text{ (R}^2=0.99), \text{ respectively.}$$

At 41 days of age, increasing levels of Zn (X) resulted in a significant linear rise on mean antibody level at 0, 12 and 120mg/kg of vitamin E, according to the equations:

$$\text{GMT}=1.8989x+2061.4 \text{ (R=0.99),}$$

$$\text{GMT}=0.2469x+2791.8 \text{ (R}^2=0.99), \text{ and}$$

$\text{GMT}=1.5692x+3180.2 \text{ (R}^2=0.84)$, respectively. For 0, 40 and 400mg/kg of Zn, there was a significant linear rise in antibody levels with the increase in vitamin E, which were expressed by the equations:

$$\text{GMT}=5,7568x+2380,6 \text{ (R}^2=0,58),$$

$$\text{GMT}=8,6303x+2396,4 \text{ (R}^2=0,79), \text{ and}$$

$$\text{GMT}=8,1861x+2808,1 \text{ (R}^2=0,99), \text{ respectively.}$$

HI Test

At 14, 28 and 35 days of age, zinc and vitamin E interaction had no significant effects on the mean antibody level. At 14 days, antibody levels increased significantly with vitamin E supplementation (0, 12 and 120mg/kg), according to the linear regression: $\text{GMT}=0.0041x+2.5595 \text{ (R}^2=0.35)$. Zinc given separately (0, 40 and 400mg/kg) had no effect on antibody levels.

In 28-day-old broilers, vitamin E and zinc supplemented separately increased antibody titers significantly according to:

$$\text{GMT}=0.1639x+45.672 \text{ (R}^2=0.57) \text{ and}$$

$$\text{GMT}=0.023x+49.513 \text{ (R}^2=0.13), \text{ respectively.}$$

Antibody levels increased at 35 days with higher vitamin E and Zn levels separately, following the quadratic equations, respectively:

$$\text{GMT}=0.3465x+79.43 \text{ (R}^2=0.71), \text{ and}$$

$$\text{GMT}=0.0468x+87.806 \text{ (R}^2=0.15).$$

Table 3 presents the mean antibody levels at each evaluation day calculated using the regression analyses of zinc and vitamin E separately.

Table 3 - Levels of hemmaglutination inhibiting antibodies expressed as Geometric Mean Titers (GMT). Results were obtained by regression analysis of Zn and vitamin E levels separately, at the indicated ages.

	GMT (HI) Age (days)		
	14	28	35
Vit E (mg/kg)			
0	2.49 (L)	43.56 (L)	75.49 (L)
12	2.68 (L)	49.98 (L)	87.97 (L)
120	3.05 (L)	65.10 (L)	120.58 (L)
Zinc (mg/kg)			
0	2.61 (NS)	47.21 (L)	84.21 (L)
40	2.71 (NS)	52.98 (L)	93.67 (L)
400	2.91 (NS)	58.45 (L)	106.14 (L)
Mean	2.74	52.88	94.67
SEM	0.051	1.092	1.166

SEM = average standard error; CV = coefficient of variation; (L) = linear model; NS = non significant.

At 41 days of age, mean antibody levels increased significantly from 0 to 120mg/kg of vitamin E, which depended on Zn isolated levels (X), according to the equations:



$GMT=0.0624x+57.035$ ($R^2=0.92$),
 $GMT=0.0389x+71.152$ ($R^2=0.58$), and
 $GMT=0.036x+95.518$ ($R^2=0.88$), respectively. Mean antibody levels showed a significant increase with higher vitamin E levels within each Zn level. For 0, 40 and 400mg/kg of Zn, mean antibody response increased linearly and significantly:
 $GMT=0.2856x+58.228$ ($R^2=0.98$),
 $GMT=0.2746x+68.513$ ($R^2=0.83$), and
 $GMT=0.2259x+82.523$ ($R^2=0.99$), respectively, in which vitamin E levels are the independent variable X.

DISCUSSION

The antibodies detected by HI and ELISA at 14 days are probably maternal antibodies that were still present in chick serum. Although the levels were statistically different, they were probably not enough to confer protection on broilers. Therefore, it might be supposed that higher levels of Zn and vitamin E delay the decrease in the maternal antibody levels.

Improved immunological response was observed at 28, 35 and 41 days old by HI. At 41 days, the interaction between Zn and vitamin E significantly affected antibody levels. Besides, zinc and vitamin E affected antibody levels individually in 28- and 35-day-old birds; antibody levels increased with increments in supplementation.

The increase in mean antibody levels was noticed by ELISA in the serum of 28-, 35- and 41-day-old birds. Besides, humoral immunological response was improved with higher levels of zinc and vitamin E both individually and in combination, except at the greater vitamin E level combined with zinc supplementation.

According to Paulillo *et al.* (1987), maximum levels of hemagglutination inhibiting antibodies were seen 21 days after vaccination. This result is corroborated by the present findings, since the highest antibody levels were detected in ELISA in the sera of 35-day-old birds, i.e., 21 days post vaccination.

Broilers supplemented with 132mg/kg of vitamin E in the diet showed improved humoral response than the control group (Tengerdy & Nockels, 1973). On the other hand, birds fed selenium and vitamin E supplemented to the diet at the levels of 0.06mg/kg and 150 IU/kg, respectively, showed significantly higher antibody levels against ND virus ten days after immunization, both in ELISA and HI (Swain *et al.*, 2000).

Leshchinsky & Klasing (2001) have reported that the effects of vitamin E on antibody production depends on antibody function. Different levels of vitamin E were

fed to broilers (0, 10, 25, 50, 100 and 200 IU/kg diet) and the humoral response against red blood sheep cells, infectious bronchitis virus and *Brucella abortus* was evaluated. The levels of antibodies against the infectious bronchitis virus and red blood sheep cells were higher in broilers fed diets supplemented with vitamin E at 25 and 50 IU/kg feed, respectively. The levels of *Brucella abortus* antibodies were not influenced by vitamin E supplementation.

Several studies have not shown better resistance against diseases, or immunocompetence, as a result of diet supplementation. It has been reported that broilers fed 100 or 250 IU vitamin E in the diet showed no differences in antibody production (Qureshi *et al.*, 1993).

Antibody production was impaired in broilers with zinc deficiency (Burns, 1983), while zinc supplementation at 8mg/kg of diet had no effects on the immunological response of young broilers (Pimentel & Cook, 1988).

Pimentel *et al.* (1991) assessed the immunological response in chicks that were fed a diet containing zinc oxide and zinc methionine. It was used the levels of 8, 18, 28, 38, 48 and 58mg/kg of zinc oxide, and 28, 38, 48, 58, 68, 78 and 88mg/kg of zinc methionine. Neither zinc source nor zinc levels affected the immune response as measured by antibody response against red blood sheep cells. These results corroborate findings reported by Kidd *et al.* (1992), who supplemented the basal diet (72mg/kg of Zn) with 80mg/kg of zinc oxide or zinc-methionine. The immune response in young broilers supplemented with different levels of zinc oxide and zinc-methionine was evaluated and showed no effects of zinc level or source on the levels of red blood sheep cells. However, the group fed with Zn-methionine showed improved humoral immunological response against *Salmonella pullorum*. Kidd *et al.* (1993) noticed that chicks originating from breeders fed higher levels of complex Zn showed better immunological response.

CONCLUSION

The interaction between the highest levels of Zn and vitamin E affected the humoral immunological response of broilers and caused a significant increase in GMT in the ELISA test.

In regard to HI results, response to immunization was improved by the highest levels of zinc or vitamin E used individually in 28- and 35-day-old birds, and also by the combination of the highest levels of these minerals in 41-day-old birds.



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