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The Effects and Interaction of Vitamin A and Zinc Food Supplements on Humoral Immune Response of Broilers to V_4-UPM (NDV) Vaccination

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Abstract: The antibody response of broilers to V_4-UPM vaccine fed diets supplemented with zinc (Zn) (0, 50, and 500 mg kg^{-1}) and Vitamin A (0, 15 and 150 mg kg^{-1}), separately or combined was evaluated. A completely randomized design was used according to a 3×3 factorial arrangement with nine treatments and four replicates per treatment. Birds were reared until 56 days old. Haemagglutaination Inhibition (HI) test was used to assess antibody levels before vaccination and at 35, 42, 49 and 56 days of age. The results were analyzed using SPSS package. The interaction between Zn and Vitamin A resulted in higher antibody levels in Group 6 at 35, 42, 49, and 56 days of age. However, interaction between the highest levels of Zn (500 mg kg^{-1}) and Vitamin A (150 mg kg^{-1}) resulted in lower antibody production in the broiler birds. The control (Group 5) showed the least level of humoral immune response. Combined Zn and Vitamin A supplementation improved antibody response to Newcastle disease virus.

Key words: Zinc, Vitamin A, interaction, newcastle disease, humoral immunity

INTRODUCTION

Newcastle Disease (ND) caused by ND Virus (NDV) which is an Avulavirus is one of the most important avian viral diseases because of its high economic impact on the poultry industry. In many tropical and subtropical countries virulent strains of ND virus are endemic (Spradbrow, 1990). In Nigeria, ND is considered one of the most important constraints for the development of profitable poultry farm and poultry-fish integrated farms in the urban and rural areas. ND is preventable through good husbandry practices and vaccination. Vaccination for protecting chickens from ND is routinely practiced throughout the world (Al-Zubeedy, 2009).

One of the several approaches to increase immune responsiveness in high intensity poultry production is to supplement rations with micronutrients (Friedman *et al*., 1998). Besides macronutrient malnutrition, deficiencies of several micronutrients also influence immune homeostasis-micronutrients like Vitamin A (retinol) and Zinc (Zn) have been shown to play different roles in host immune response (Karamouz *et al*., 2010; Monoura *et al*., 2008; Cardoso *et al*., 2006). The results of several experiments and clinical studies suggest an interaction between Zn and Vitamin A (Christian and West, 1998; Brow *et al*., 1976). The present study was undertaken with a view to evaluating the effects and interaction of Vitamin A and zinc food supplements on immune-response of broiler chickens vaccinated with V_4-UPM vaccine.

MATERIALS AND METHODS

Experimental animal: A total of 36 unvaccinated day old Anak broiler chicks were purchased within 24 h of hatching from Agrited chicks, Awka, Anambra State, Nigeria. The chicks were initially reared together in a pen fitted with electric bulbs and the open sides of the pen covered with polyethylene sheets to provide warmth. The birds were fed with commercial starter mesh (Guinea feed, Benin City, Nigeria) which contained 0.19 Crude Protein (CP) and 2500 kcal kg^{-1} Metabolisable Energy (ME) for 14 days.

After 14 days, the birds were fed cassava meal containing cassava and fish meal (CP = 0.22, ME 2960.4 kcal kg^{-1}) for 7 days. This was to wean the birds of any Vitamin A and zinc micronutrients in their system. Apart from being unvaccinated, the birds were neither given coccidiostat nor antibiotics. Strict hygienic husbandry was practiced as a means of preventing disease occurrence. At the end of the weaning period, the 21 days old chicks were tagged and randomly assigned to groups.

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Experimental design: A randomized design was used according to a 3×3 factorial arrangement with nine treatments and four replicates per treatment. There were three levels of zinc 0, 50 and 500 mg kg^{-1}) in form of zinc oxide and three levels of Vitamin A as retinyl palmitate (0, 15 and 150 mg kg^{-1}). Therefore, nine diets (representing nine groups) were prepared with the following levels of zinc and Vitamin A per kg of Guinea starter and finisher feed used:

- Group 1 = 50 mg Zn
- Group 2 = 500 mg Zn
- Group 3 = 15 mg Vitamin A
- Group 4 = 150 mg Vitamin A
- Group 5 = No zinc or Vitamin A
- Group 6 = 50 mg Zn and 15 mg Vitamin A
- Group 7 = 500 mg Zn and 15 mg Vitamin A
- Group 8 = 50 mg Zn and 150 mg Vitamin A
- Group 9 = 500 mg Zn and 150 mg Vitamin A: All birds were supplemented until 56 days of age

The virus strain used for the investigation: The seed was a lentogenic NDV strain V_4-UPM obtained from Professor AiniIderis of the Faculty of Veterinary Medicine and Animal Science, University of Pertanian, Malaysia (UPM). The virus was propagated in embryonated hen eggs and the stock virus titrated and stored as described by Echeonwu *et al*. (2008).

Vaccination: The 21 days old birds were administered food supplements for another 7 days before vaccination. At 28 days of age, birds were screened by HI test technique for NDV antibody prior to initial vaccination orally with 2 drops of standard antigen titre of 4 Haemagglutinating Unit (4HAU) = 1/64. Blood samples from test and control were collected at 35th, 42nd, 49th and 56th days of age. All the blood samples were obtained by wing vein punctured and subjected to Haemagglutination Inhibition (HI) test.

Analysis of data: Statistical Package for Social Science (SPSS) Version 16:0 programme was used for analysis of data.

RESULTS AND DISCUSSION

Pre-vaccination HI test result showed an HI titre of zero for each bird examined. This implies that the birds have not been previously exposed to NDV. The results of antibody levels in the sera of broilers (GMT) obtained in HI after vaccination are shown in Table 1. Antibody levels in all samples were significantly affected ($p<0.05$) by both Zn and Vitamin A and also by the combination between them. At 35 days, Vitamin A and zinc supplementation separately increased antibody titers significantly according to the linear regression:

Table 1: Geometric Mean Titres (GMT) of antibodies against the Newcastle disease virus assessed by HI in the sera of broilers at 35th, 42nd, 49th and 56th days of age

Vitamin A (mg kg^{-1})	Zinc (mg kg^{-1})	GMT (HI) broilers age (days) 35	42	49	56
0	0	6.25	7.25	9.00	6.25
	50	8.75	10.00	9.00	6.75
	500	7.50	9.50	11.25	8.75
15	0	9.75	11.00	12.25	9.25
	50	13.50	15.75	18.50	15.50
	500	10.25	11.50	14.50	9.75
150	0	11.00	11.50	14.25	10.00
	50	12.50	14.75	16.75	14.00
	500	10.25	11.25	12.00	9.25
SEM		1.24	1.53	1.66	1.86

SEM = Standard Error of the Mean

$$GMT = 7.78 + 0.0218X\ (R^2 = 0.44)$$

And:

$$GMT = 9.95 + 0.0427X\ (R^2 = 0.88)$$

Antibody levels increased at 42 days with higher Vitamin A and zinc levels supplementation separately. At 49 days of age, mean antibody levels increased sinificantly for 50 mg kg^{-1} of zinc which depended on Vitamin A as the independent variable (X) according to the equation:

$$GMT = 8.91 + 0.04X\ (R^2 = 0.99)$$

At 56 days of age, a reduction in serum antibody titre was observed in all the birds. The results obtained in this study have a close relationship to the findings of Sklan *et al*. (1994) who observed that the immune response of chicks were higher when feed with addtional Vitamin A in their normal ration compared addition Vitamin A deprived group. This may be due to the role of Vitamin A on the proliferation or activation of B lymphocytes. Cardoso *et al*. (2006) that showed that vitamins and minerals (Vitamin E and zinc) increment increased mean antibody levels in broilers vaccinated against Newcastle disease. Swain *et al.* (2000) recorded that vitamins and minerals increased mean cellular and humoral immunity in broilers.

Humoral immunological response was improved sinificantly with increasing levels of zinc and Vitamin A in combination at 49 days post vaccination however, higher levels of both zinc (500 mg kg^{-1}) and Vitamin A (150 mg kg^{-1}) induced lower antibody response. This result is related to the findings of Friedman *et al*. (1998)

that observed that in chicks receiving 150 mg kg^{-1} Vitamin A, antibody production was significantly lower. These results indicate that humoral immune responses are directly affected by Vitamin A and zinc and that excessive intake of them has detrimental effect on antibody production in broilers.

Zinc and Vitamin A supplementation had a significant positive effect on the antibody levels in the experimental birds and the effect of combined supplementation was additive. This is possible because of the role of zinc in bioavailability of Vitamin A. Burns (1983) demonstrated that antibody production was impaired in broilers with zinc deficiency. However, Rahman *et al*. (2002) showed that combined zinc and Vitamin A improves immune response in human children.

CONCLUSION

This study showed that interaction between zinc and Vitamin A affected the humoral immunological response of broiler to V_4-UPM (NDV) vaccination.

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