Pak. j. life soc. sci. (2003), 1(1): 13-16

Pakistan Journal of
Life and Social Sciences

Effect of Vitamin-A Deficient Diet on Immune Response in Newcastle Disease Infected Broilers

Farzana Rizvi, Kashif Asghar and M.A. Hafeez[1]
Department of Veterinary Pathology, University of Agriculture-Faisalabad
[1]Department of Veterinary Parasitology, University of Agriculture, Faisalabad-Pakistan

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Abstract

Effect of vitamin A deficiency was determined on the immune response of broilers. The experimental feed was prepared having vitamin A level at the rate of 500 IU/kg of feed. Vitamin A deficiency in feed significantly lowered (P<0.05) the gain in body weight with poor feed conversion ratio (FCR). In Newcastle disease virus (NDV) infected broilers kept on vitamin A deficiency showed greenish white droppings, ruffled feathers with leg weakness, while NDV infected birds kept on vitamin A at the rate of 1500 IU/kg of feed (Standard requirement) did not show clinical signs of ND. Geometric mean titer (GMT) against NDV was lower in broilers kept on vitamin A deficiency in feed.

Key words: Newcastle disease, Vitamin A, broilers immune response,

Introduction

Newcastle disease is a contagious infectious disease of poultry and its outbreaks have been reported even in vaccinated flocks (Irfan and Ashfaque, 1985). Occurrence of disease in vaccinated flock may be due to failure or inadequate antibodies production against the Newcastle disease virus. The later may be due to the deficiency of vitamins and minerals in feed. Vitamin A, known as anti-infection vitamin (Slinger, 1984), has an important role in health and function of epithelial lining of gastrointestinal, respiratory and genitourinary tract of birds and animals.

The minimum requirement of vitamin A for broiler chicken from one day to seven weeks is 1500 IU per kilogram of the diet. Deficiency of vitamin A may occur due to improper mixing of feed, impaired absorption from intestine due to coccidiosis and worm infection and instability of vitamin A preparations. Vitamin deficiency is associated with increased susceptibility of infection and defects in both maternal and acquired defense mechanisms (Suskind, 1977).

Corresponding author: Farzana Rizvi
Department of Veterinary Pathology
University of Agriculture, Faisalabad-Pakistan.
E.mail: frizviali@hotmail.com

The invading infectious agent itself can also affect host resistance (McChesney and Oldstone, 1987). The effect of vitamin A deficiency and infection are synergistic. This relationship has been observed in vitamin A deficient chickens infected with Newcastle disease virus (Bang *et al*., 1973).

Keeping in view the importance of vitamin A in maintenance of mucous membrane and production of immunity against infections, a study was designed to determine the effect of vitamin A deficiency in diet on immune response in Newcastle disease virus infected broiler chicks.

Materials and Methods

A general survey was conducted and feed samples from three different feed mills were collected and level of vitamin A was determined in them, following the method as described by Anonymous (1990).

Sixty, day-old-broiler chicks were procured from a commercial hatchery (Big birds, Lahore). The chicks were randomly divided into two groups (A and B) each comprising of 30 birds and birds from both groups (A & B) were subdivided into subgroups (A_1 & A_2 and B_1 & B_2). The broilers of group A_1 and B_1 were vaccinated against ND at the age of 7^{th} and 23^{rd} day intraocularly and orally, while group A_2 and B_2 was kept as unvaccinated. The chicks were kept in cages under standard management conditions. All the birds in group A were provided with vitamin A deficient feed (500 IU/kg) and water *ad libitum.* The birds of group A_2 and B_2 were experimentally infected with ND virus at the rate of $10^{-4.81}$/0.1 ml of virus at the age of 31st day through intra ocular route.

Preparation of Vitamin A deficient feed

Experimental vitamin A deficient feed was prepared following the method described by West *et al.* (1992). Composition (g/Kg) of the feed is given as under.

White maize	340
Sorghum	160
Soya-bean flour	140
$CaCO_3$	51.5
Blood meal	25
Fish meal	25
$CaHPO_4.2H_2O$	15
Soya-bean oil	10
Vitamin-mineral premix	10
NaCl	2.5
DL-methionine	2

L-lysine hydrochloride 2

Collection of blood

About 4-5 ml of blood was collected without anticoagulant from wing vein of 7 randomly selected birds from each group at 15 and 30 days of age. The blood was used for serum separation for determination of antibody titer against NDV.

Determination of vitamin A in serum

Vitamin A concentration in serum was determined by the spectrophotometric method (Neelds and Pearson, 1963).

Immunological studies

Serum was used for the determination of antibody titer against Newcastle disease virus following the method described by Buxton and Fraser (1977).

The data was analyzed by using one-way analysis of variance technique and means were compared by using least significant difference (LSD) test.

Results

Feed samples from three different mills were collected and concentration of vitamin A in them was as below.

Feed A = 1100 IU/kg of feed
Feed B = 1350 IU/kg of feed
Feed C = 700 IU/kg of feed

Thirteen birds of group A_2 showed loss of condition, less growth, drowsiness, in-coordination. Paralysis of legs and ruffled plumage was observed in seven chicks. While the birds of group B_1 & B_2 did not exhibit any deviation from normal behavior and there was no mortality.

Live body weight of experimental broilers is presented in Table 1. There was non- significant effect of vitamin A deficiency on weight of broilers upto the age of 3 weeks. From 4th week onward, there was significant ($P<0.001$) decrease in body weight of broilers of vitamin A deficient groups.

The effect of vitamin A deficiency on feed conversion ratio is shown in Table 2. In the 1st week, feed conversion ratio for all the groups was almost same. FCR of broilers of vitamin A deficient group was poor as compared to the control group from second week onward.

There was significant decrease in vitamin A concentration in the serum of birds of groups A_1 and A_2 as compared to control group (B) at the age of 15, 28 and 38 days as shown in Table 3.

On second day post-infection, three birds in vaccinated plus infected group (A_1) showed anorexia, greenish white droppings, leg weakness and ruffled feathers and three birds died on 3rd day post-infection. On 4th day post-infection, sickness with greenish white droppings and pasting on vent was observed in two birds. On 5th day post-infection one bird died. Paralysis and ruffled feathers were observed on 6th day post-challenge. One bird died on 7th day, while five birds remained healthy.

In non-vaccinated plus infected group (A2) on day 1st and 2nd day post inoculation, two birds were off feed having greenish white droppings. Respiratory signs were observed in one bird and two were paralyzed on 3rd day post infection and one of them died same day. Two bird were paralyzed with respiratory problem on 4th day post infection. Death was also observed in one bird. Two birds were died on 5th day post challenge. Up to 7th day, two birds showed sickness and were off feed having respiratory problems and leg weakness. Two birds died on the same day. The birds of control group (B) were remained healthy, seven birds of non-vaccinated group showed signs of diarrhea, while vaccinated birds did not show signs of disease.

At 15 and 28 days of age GMT was decreased in unvaccinated broilers kept on vitamin A deficient feed as compared to control group (B).

Discussion

In the present study the birds offered vitamin A deficient (500 IU/kg of feed) feed had significantly lower mean body weights than the control (1500 IU/kg of feed). Sijtsma *et al.* (1989) and Nockel *et al.* (1984) also observed decreased body weight in broilers kept on vitamin A deficient feed. Whereas, Sijtsma *et al*. (1990) observed non-significant difference in body weights of broilers kept on vitamin A deficient feed as compared to the control.

The feed conversion ratio was significantly lower in vitamin A deficient broiler chicks as compared to control group. The findings are in agreement with those of Sijtsma *et al*. (1989a) and West *et al*. (1992) who reported that diets free of vitamin A can produce sudden and uncontrollable vitamin A deficiency with consequent loss of appetite and malabsorption of protein. The decrease in body weight of broilers and poor FCR indicated that vitamin A deficiency interfere in proper utilization of feed. Uni *et al*. (1998) reported that vitamin A deficiency caused hyper-proliferation of enterocytes, reduced villus height and decreased in alkaline phosphates activity. Hyper-proliferation of enterocytes interferes in absorption and thus lead to poor weight gain.

The clinical signs in experimentally vitamin A deficient birds prior to infection were loss of condition, less growth, drowsiness, incoordination, ruffled plumage and paralysis of legs. Rao (1988) also observed poor growth, weakness, muscular incoordination and ruffled plumage in vitamin A deficient broilers. Anorexia, greenish white diarrhoea, leg paralysis and respiratory signs were observed after the NDV infection. Mishra *et al*. (2000 and 2001) and Alexander (1980) observed similar signs after the NDV infection. However, torticollis and twisting of neck was also observed by Benerjee *et al*. (1994).

GMT against NDV of vitamin deficient birds was lower as compared to control group. Similar observations were obtained by Davis and Sell (1989). Herlyn *at al*. (1975) and Sklan *et al*. (1994) reported a negative influence of vitamin A deficient diet on immune response.

From this study it was concluded that deficiency of vitamin A depressed the growth of broilers. The decrease in body weight was due to poor FCR with loss of appetite. Feed deficient in vitamin A increased the severity of disease following NDV infection by decreasing the production of antibodies against the virus.

Table 1: Body weight (gm) of broilers kept on vitamin deficient feed

Age in weeks	Control Group B	Vitamin A deficient	
		Vaccinated	Unvaccinated
1	149.14 ±7.27	133.85 ±6.66	133.57 ±4.04
2	253.71 ±13.87	233.42 ±10.30	247.28 ±8.46
3	363.57 ±21.23	301.57 ±15.88	325.00 ±20.88
4	433.57 ±15.50	337.143*** ±15.69	342.71*** ±18.11
5	1110.72 ±60.96	739.28*** ±43.45	810.00*** ±30.00

Each figure represents mean (± standard error of the mean) of seven chicks. Data subjected to analysis of variance revealed significant differences among the three groups.
***Significant difference (p<0.001) compared with the control.

Table 2: Feed conversion ratio in broilers kept on vitamin A deficient feed

Time Interval (Weeks)	Control (Group B)	Vitamin A deficient	
		Vaccinated (Group A1)	Unvaccinated (Group A2)
1	0.90	0.89	1.03
2	1.17	1 .27	1.25
3	1.18	1.29	1.39
4	1.23	1.27	1.29
5	0.64	0.69	0.70

Table 3: Concentration of vitamin A in serum of broilers kept on vitamin A deficient feed

Age in days	Control (Group B)	Vitamin A deficient (500 IU/kg)	
		Vaccinated (Group A1)	Unvaccinated (Group A2)
15	1.65±0.06	0.60±0.01**	0.59±0.01**
28	1.67±0.06	0.56±0.01**	0.54±0.02**
38	1.71±0.05	0.28±0.02**	0.24±0.02**

Each figure represents mean (± standard error of the mean) of seven chicks. Data subjected to analysis of variance revealed significant differences among the three groups.
**Significant difference (p<0.001) compared with the control.

Table 4: Geometric mean titer against NDV of broilers kept on vitamin A deficient feed

Days	Control		Vitamin A deficient feed	
	Group B1	Group B2	Group A1	Group A2
15	170.85	7.43	115.88	5.25
28	190.72	6.20	150.23	4.11
38	165.45	_	132.84	_

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