

Effects of Vitamins E and C Supplementation on the Immune Response of Broiler Chicks

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Abstract

The present study was done to know the effects of supplementations of vitamin C and E on humoral immune response against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) and on lymphoid organs in broiler birds. One hundred and twenty day old chicks were purchased from a local hatchery and were reared in an open house shed. On day 5th all the chick divided randomly into 4 groups A, B, C and D (30 birds in each). On the day 5th and 11th, the chicks were vaccinated against ND and Disease IBD. Booster doses of both vaccines were given on day 28. The chicks were offered Vitamin E (600 mg l⁻¹), Vitamin C (600 mg l⁻¹) and Vitamin E+C (300 mg l⁻¹ each), for 5 consecutive days in drinking water on day 5 and 28. Weekly serum Hemagglutination Inhibition (HI) antibody titers against ND virus, total body weight, feed conversion ratio (FCR) and weight of lymphoid organs were recorded until the day 49. Geometric mean HI antibody titers against ND remained maximum in group C. Statistical analysis revealed a non significant (P<0.05) differences among the various treatment groups in weight gain. At day 49, the total weight gain was maximum in group C (2196.0 gm) followed by group A (2155.0 g), group B (2146 g) and group D (2094 gm). The feed conversion ratio was the best in the group B (1.66) followed by group C (1.69) with a non significant difference. From the study, it was concluded that the combined effect of Vitamin E+C was better as compared to separate supplementation of Vitamin E and Vitamin C.

Keywords: Broiler chicks; Vitamin E; Vitamin C; Newcastle disease; Infections bursal disease; Antibodies; Hemagglutination inhibition; Weight gain; Feed conversion ratio; Lymphoid organs; Pakistan

Introduction

Modern commercial broilers have compromised immunocompetence, higher mortality and lower resistance to stresses. In the literature, a number of outbreaks were reported in the vaccinated flocks despite vaccination against Newcastle Disease (ND), due to improper immune response and vaccine failures [1]. Outbreaks mostly in non-vaccinated broilers and layers, causing 100% morbidity and 25% mortality were reported due to managemental problems, which include poor Biosecurity, imbalanced ventilation, extreme ambient temperatures, stress factors, some local and imported substandard vaccines and medicines [2]. Irrational use of antibiotics and poor quality of compound poultry feed has cumulatively made the birds vulnerable to the attack of various infectious diseases [3]. Vaccine failure and disease prevalence may be attributed to immunosuppression. Immunosuppressive flocks generally experience an increase incidence of opportunistic infection and respond poorly to routine use of vaccines. Proper vaccination and monitoring of the post vaccination immune response are important ways to control ND and IBD diseases [4]. Immuno-stimulation includes a prophylactic and therapeutic concept to stimulate nonspecific and specific immune response [5]. Many immune-stimulating substances that have been used in poultry with success are Levamisole, Vitamin E and Selenium [6-8], Ascorbic acid and Vitamin D [9,10]. Vitamins have been

established as the basis of disease control programs in the poultry industry. Among these, Vitamin C and E in the context of their significance as a proficient immune stimulant and immune system regulators, has always been a subject of prime interest. Vitamin E is a natural antioxidant for cellular membranes and regulates the production of prostaglandins and leukotenes [11], which minimizes the damage resulting from the cytotoxic action in organisms and improves the phagocytic activity of macrophages in young birds [12]. The chicks are capable of biosynthesis of ascorbic acid, but this ability became inadequate under stress condition, such as in high environmental temperature, high humidity, a high production rate and parasitic infestation [13].

Vitamin E and C act synergistically. Vitamin E enhances antibody production and Vitamin C enhances macrophage activity, hence it plays an important role in enhancing both cell mediated and humoral immunity in broiler chicks, while Vitamin E and C in combination enhances the immune response and performance of broiler [14]. Although there have been numerous studies to determine the effect of a single Vitamin on the immune system, by using them with live NDV, but scanty literature is available on their combined effects.

The objectives of the study were to determine the effects of supplementation of Vitamin E and C separately and in combination through drinking water on the humoral immune response in broiler chicks against NDV. The performances of chicken were evaluated through antibody titers, weekly body weight gain, feed consumption, FCR and lymphoid organ weight.

Materials and Methods

The present study was conducted in an open house poultry shed at Faisalabad, Punjab, during the month of June and July, 2016. A total of 120 one day old broiler chicks was purchased from a local hatchery (Noor chicks Faisalabad). These were randomly divided into 4 groups (A-D) consisting of 30 birds in each. All the chicks were raised under similar standard management and housing conditions. Chicks of all groups were primed with ND and infectious bronchitis disease vaccine (Bioteke. Italy) on day 5, and IBD (Bioteke. Italy) live vaccine, on day 11 orally. At day 17 birds were vaccinated against Hydro pericardium Syndrome vaccine (Sanna Labs. Faisalabad). The birds were boosted with both ND and IBD vaccines on day 28 in drinking water. Vitamin supplementation in different groups was given orally for 5 consecutive days starting from day 5 and then day 28. The chicks of group A were

Pharma) @ 3 ml⁻¹ (600 mg.l⁻¹). The chicks of group B were supplemented with Vitamin C (ascorbic acid 20% solution) @ 3 ml⁻¹ (600 mg.l⁻¹) and group C was supplemented with Vitamin E+C combination (C. 20% and E. 20%) @ 1.5+1.5 ml.l⁻¹. The chicks of group D were kept as control.

Collection of samples and HI assay

Blood samples of 5 chicks from each group were collected at 1, 7, 14, 21, 35, 42 and 49 days of age via cardiac puncture by inserting 24 gauge needles fitted with 5 ml plastic syringe parallel to the sternum just beneath the first sternebra. Care was taken not to puncture the lungs. The syringes were kept in slanting position at room temperature for 4-5 hours and then serum was separated and stored at -20°C till use.

Antibody titers against NDV in sera were carried out through HI assay at disease section poultry research Institute Rawalpindi [15].

Feed conversion ratio

A weighed quantity of feed was offered to each experimental group twice a day throughout the experimental week and the left over; feed was weighed to determine weekly feed consumption by each group. At the end of the experiment (7 weeks), feed conversion ratio (FCR) of each group was calculated by the following formula [16].

$$FCR = \text{Feed consumption} / \text{Body weight gain}$$

Body and lymphoid organs wright

Five chicks were weighed on day 1 and then on a weekly interval in all groups. At the end of the experiment (49 days) five birds from each group were killed and lymphoid organs (Bursa of Fabricius, thymus and spleen) were removed carefully after removal of the fat and tissue debris. The organs were grossly examined to detect any gross pathological change. These organs were then weighed separately.

Statistical analysis

Haemagglutination Inhibition titers were converted into geometric mean titers (GMT) for each group [17]. Least significant Difference (LSD) test was applied on FCR. Statistical differences were considered significant above 95% probability using SPSS 15.0 software.

Results

The antibody titers against NDV were determined on day at 1, 7, 14, 21, 35, 42 and 49 (Table 1)

Age days	in Vaccination Against ND	Geomean Antibodies Titers in Expt. Groups			
		A (Vit. E)	B (Vit. C)	C (Vit. E+C)	D (Control)
1	-	53.0	53.0	53.0	53.0
5	Priming + Vit. Supp	-	-	-	-
7		177.8	175.3	180.8	125.1
14		168.1	165.1	179.2	150.6
21		160.6	230.1	251.1	155
28	Booster + Vit. Supp	157.8	160.8	161.8	125
42		125.8	79.1	157.2	38.8
49		89.1	44.6	125.8	31.6

Table 1: Geometric mean HI antibody titers against the LaSota strain of NDV in treated and control broiler chicks

The GMT values were similar in all the groups at the age of the day 1 (GMT 53.0). At day, 7th titers were 177.8, 175.3, 180.8 and 125.1 in groups A, B, C and D respectively. This indicated a higher immune response in group C. On day 14th GMT values were at the highest level (179.2) in Vitamin E+C supplemented group (C), followed by (168.1) in group A and 165.1 in group B, whereas the lowest HI titers (150.0) were in the control group (D). Similarly GMT values were at the highest level (251.1) in group C and followed by group B (230.1), group A (160.6) and group D (155.0), respectively at day 21. At days 42 and 49, similar patterns of immune response prevailed as the highest GMT was observed in group C. More increase in GMT antibody titers in group C than all other groups were observed throughout the study.

During this study, the body weights of chicks of groups A, B and C were more than chicks in control group (Table 2).

Age (days)	Groups (mean body weight ± standard deviation)			
	A (Vit. E)	B (Vit. C)	C (Vit. E+C)	D (Control)
1	44.2 ± 2.85	44.2 ± 4.57	44.6 ± 5.31	44.4 ± 3.72
7	71.2 ± 4.94	72.2 ± 11.86	72.2 ± 5.74	76.6 ± 9.36
14	99.2 ± 9.07	98.0 ± 3.63	97.6 ± 3.72	95.0 ± 4.60
21	172.4 ± 2.65	175.6 ± 6.46	171.0 ± 3.84	166.8 ± 6.52
28	645.6 ± 50.61	594.6 ± 65.05	648.4 ± 56.01	612.0 ± 15.84
35	1054.8 ± 46.82	1016.6 ± 19.34	1014.8 ± 15.52	955.0 ± 50.59
42	1806.0 ± 122.75	1788.0 ± 94.26	122.0 ± 112.283	1712.0125.45

49	2155 ± 202.40	2146 ± 91.15	2196.0 193.00	±	2094.0 131.02	±
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Table 2: Body weights of broiler chicks supplemented with vitamins E and C and in combination.

Statistical analysis revealed no significant differences among the various treatment groups. The results of net weight gain on day 49 in groups A, B, C and D showed maximum (2196.0) in group C followed by 2155, 2146.0 and 2094 in group A, D and B, respectively.

Feed conversion ratio (FCR) in treatment groups A, B and C is significantly better than that of group D (Table 3).

Groups	Total feed consumed (Kg)	Total body weight (Kg)	FCR
A	402.9	223.73a	1.80a
B	406.6	244.35b	1.66b
C	407.95	240.57b	1.69b
D	408.91	216.44	1.88c

Means followed by the same letters do not differ significantly ($P \leq 0.05$)

Table 3: FCR of chicks in various treatment groups at day 49

However, FCR of group B and C differ non significantly with each other.

The results of the present study showed higher weights of Bursa of Fabricius, spleen and thymus in group C than in group A, B and D (Table 4)

Lymphoid Organs	Groups (mean ± stander deviation)			
	A	B	C	D
Bursa of Fabricius	1.51 ± 0.04	1.47 ± 0.07	1.60 ± 0.01	1.35 ± 0.04
Thymus	7.79 ± 0.02	7.70 ± 0.11	7.86 ± 0.04	7.50 ± 0.02
Spleen	4.65 ± 0.08	4.60 ± 0.10	4.757 ± 0.10	4.51 ± 0.02

Table 4: Lymphoid organ weight (g) at 49 days of age of broiler chicks fed treatments

Discussion

Antibody titers against ND

The study was carried out to observe the effects of supplementation of Vitamin E and C separately and in combination. The results revealed that both the Vitamins have beneficial effects on immune response when used separately or in combination (Table 1).

The raised GMT of HI antibody titers was observed in the treatment groups than in the control group. Fall in GMT of HI antibody titers in all groups on day 28 onward might be due to the suppression of humoral immunity against NDV with IBD (because in this study ND and IBD vaccines were used on the same day [18]). The results of this study are also in correlation with the earlier findings [19]. Vitamin C having antioxidant property protects immature lymphocytes from damage by free radicals and enhances immune response which results

in increases antibody titers against ND virus upon supplementation with different levels of ascorbic acid [20,21]. Vitamin C under heat stress and disease conditions protects the birds from extreme conditions and improve the immune response [22,23]. The supplementation of combination of Vitamin C and E in group C in lesser doses showed better results to improve humoral immune response against NDV than individual supplementation of these Vitamins. Vitamin E enhances antibody production and Vitamin C enhances macrophage activity, thus act synergistically [14,24].

Body weight gain and FCR

The results of the present study showed that there was increase in body weight in broilers when they were fed a Vitamin supplemented diet [21,24,25]. Although the highest weight gain was observed in group C but there was not much difference in group A and B. The highest body weight gain at 42 days ($P < 0.05$) indicating an additive effect of Vitamin E and C [14]. Feed conversion ratio (FCR) depicted in treatments groups A, B and C is significantly better than that of group D (Table 3). However, FCR of group B and C differ non-significantly with each other. These findings coincide with that of earlier findings in literature, as with the supplementation of vitamin C, improved FCR of broilers during heat stress [9, 21, 26, 27].

Weight of lymphoid organs

The Bursa of Fabricius, spleen and thymus are main lymphoid organs in birds and their weights are directly related to immune systems and protection. In this study, the results showed higher weights of Bursa of Fabricius, spleen and thymus in group C than in group A, B and D (Table 4). The chick fed on Vitamin E+C had significantly ($P < 0.05$) higher spleen and thymus ratio than individual supplementations of vitamin E and vitamin C, which also improved the weight of the spleen, thymus and bursa than control [14].

It remains unclear whether the beneficial effects of this Vitamin intake on immune organs of chicken occur through a direct Vitamin lymphoid organ interaction or through indirect effects caused by changes in Neuro endocrine status. The protective effects of Vitamin E and C on lymphoid organ weighs may partially be a result of reducing circulating levels of glucocorticoids.

Conclusions

This study concluded that the supplementation of Vitamin E, C or E +C combination in drinking water at the time of vaccination against NDV improve humoral immune response, body weight and FCR. However, supplementation of Vitamin E+C in combination gives better humoral immune response against NDV. The exact nature of *in vivo* interaction and synergistic effect of Vitamin E and C on the immune function needs further investigations.

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