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Immuno stimulatory effect of dietary supplementation of zinc sulphate and zinc-methionine on immune response in broilers

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ABSTRACT

A study was conducted on 288 broiler male chicks fed with maize-soybean-fishmeal based diet supplemented with Zinc-sulphate at 0(T_1), 20 (T_2), 40 (T_3), 60 (T_4) and 80 (T_5) mg/Kg diet and Zinc-methionine (BIOPLEX) at 20 (T_6), 40 (T_7), 60 (T_8), 80 (T_9) mg/Kg diet. Each treatment consisted of 4 replicates with 8 birds per replicate. All the birds during the first week were fed with a basal diet without any zinc supplementation to deplete the body zinc reserves. From 1st to 4th week the birds were fed with starter diet containing 23.0% CP, 2877 Kcal of ME/Kg diet and 5th and 6th week the birds were fed with finisher diets containing 20.4% CP, 2943 Kcal of ME/Kg diet. The zinc supplementation showed positive significant (P<0.05) immune response to SRBC titers, NDV titers and CMI index indicating that there is increased immune response to zinc supplementation. The Zinc-methionine supplementation showed better immune response than Zinc-sulphate supplementation. The SRBC titers and ND titers showed better humoral immune response with supplementation at 60 mg/Kg diet and 80 mg/Kg diet respectively with both Zinc-sulphate and Zinc-methionine. The CMI index indicated that the cell mediated immune response was highest at 40 mg/Kg dietary zinc-sulphate and 80 mg/Kg dietary Zinc-methionine supplementation. The heterophill to lymphocyte ratio indicated that birds showed less stress when zinc was supplemented.

Key words: Zinc, Zinc Methionine, Immune response, Broilers

INTRODUCTION

Zinc (Zn) is an essential trace element in all living systems by actively involving in enzyme function, maintaining immune response, energy production, protein synthesis, stabilization of membranes against bacterial toxins, antioxidant enzyme production, maintenance of lymphocyte replication and antibody production. Its deficiency may lead to retarded growth, shortening, and thickening of bones scaling of skin, reduced feed efficiency and even death in severe cases [1, 2, 3, 4]. It is essential to the integrity of the immune system, in cation-anion exchange (and therefore water balance) as well as in the maintenance of normal vitamin A concentration in plasma and in ovarian function. [5] reported enhanced antibody responses to sheep red blood cells (SRBC) and increased mitogen stimulation by Phytohaemagglutinin-P (PHA-P)

An attempt was made to present the critical information concerning the present investigation which is intended to know the effect of dietary supplementation of zinc sulphate and zinc-methionine on immune response in broilers. The main objective of interest in supplementing Zn in the diets of the broilers is that, it has got immuno stimulatory effect.

MATERIALS AND METHODS

The experiment was performed with 288 day old broiler male chicks which were procured from the local hatchery and were distributed randomly into nine treatments with 32 birds per treatment (8 birds per replicate with four replicates per treatment). Nine treatments (T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8 and T_9), with four replicate groups of 8 chicks in each were allowed ad libitum access to the experimental diets and fresh potable water. Chicks were maintained in heated thermostatically controlled stainless steel batteries with raised wire floors. To minimize Zn contamination from the environment, stainless steel feeders and waterers were also used. Chicks were exposed to constant fluorescent light 24 hour per day. Nine treatments were prepared using corn-Soya bean based diets using BIS specifications with inorganic Zinc sulphate at 0, 20, 40, 60, 80 mg/kg diet (T_1, T_2 , T_3, T_4 , and T_5) and organic Zinc-methionine at 20, 40, 60 and 80 mg/kg diet supplementation (T_6, T_7, T_8, and T_9). The first control treatment (T_1) with 4 replicates was fed on basal diet without zinc supplementation. The T_2, T_3, T_4, T_5 were fed on basal diet with 20, 40, 60, 80 mg/kg diet Zinc Sulphate and T_6, T_7, T_8, and T_9 were fed on basal diet with Zinc-methionine supplementation. The basal diet was prepared using corn-soy bean, fish meal based diet and it was mixed with the inorganic zinc sulphate and organic Zinc-methionine as per the concentrations described above. The organic Zinc-methionine (BIOPLEX) was procured from Vet-care India private limited. The mineral mixture was prepared using the feed grade mineral supplements as per the BIS (1992) specifications and was mixed in the basal diet along with vitamins and feed additives.

For the first 7 days all the birds were fed with basal corn soy based diet so that the body Zinc levels are depleted. On the day 8, the chicks were weighed individually and the birds averaging from 128 to 135 gm were distributed randomly into 36 cages (4 replicates / treatment with each cage having 8 birds). Four birds from were selected at random at 37 days and were injected intravenously with 0.5% SRBC (Sheep Red Blood Cells, a T-cell dependent antigen; [6]). Sheep blood was collected in EDTA added vial. After centrifugation at 5000 rpm for 5 min and cells were washed 3 times in a normal saline solution (9 gm/l) and diluted to 0.5% SRBC solution. The birds were immunized with a dose of 0.1 ml intravenous (0.5%). The blood from the immunized birds was collected 5 days after post immunization and Haemagglutination assay is done .The same procedure (for preparation of 0.5% SRBC) was used to collect SRBC from the same sheep and was diluted to 0.75% solution. Total SRBC antibody titers were assayed in the plasma of each bird according to the agglutination method described by [7]. Anti-body titers were reported as Iog_2 of the highest dilution of plasma that agglutinated an equal volume of a 0.75% SRBC in a solution of normal saline (9 gm/l).The procedure for the haemagglutination inhibition (HI) test consists of a standard amount of virus added to serial fold dilutions of the test serum (Beta method). 50 µl of normal saline was added in all the wells and 50 µl of the serum was added in the first well and made two fold dilutions. 50 µl of the suspension is discarded after thorough mixing of the contents from the last well. To this 50 µl of virus (NDV) containing 4 HA units was added in all the wells and left for 20 min. About 50 µl of 1% chicken RBC was added to all the wells and left for 45 minutes incubation. Controls for virus, RBC and serum are kept. The titer is calculated based on the end point (HI titer of the serum) which was the reciprocal of the highest dilution that shows 100% inhibition of haemagglutination.

The cell mediated immunity was studied using PHA-P antigen. Two birds from each replicate were taken at the end of 42 days and the initial thickness (To) of the wattle is measured and was injected with 0.01 ml of PHA-P antigen (Gennie laboratories, Bangalore) in to the wattles of each bird and the wattle thickness was measured at the end of 24 hrs (T_{24}). The extent of thickness is taken in to consideration to estimate the cell mediated immune response. The cell mediated immunity was assessed by measuring the hypersensitivity response of cutaneous basophills (CBH) to phytohaemagglutinin-P (PHA-P).The wattle thickness is measured with constant tension micrometer prior to T_0 and after T_{24} of injection. The CMI index is calculated using the following formula.

$$\text{CMI index}=\frac{[\text{final thickness-initial thickness}}{\text{final thickness}}\times 100$$

Heterophill to lymphocyte ratio

A total of 72 birds (2 birds from each replicate) were tested for leukocyte ratio at 6 weeks of age. The selected birds were carried to a separate room and blood was collected immediately. Two drops of blood was taken from a small puncture in the comb of each bird and one drop was smeared using Giemsas stain and kept for approximately 2 to 4 h after preparation by methyl alcohol fixation. One hundred leukocytes, including granular heterophills, eosinophills, and basophills and non granular lymphocytes and monocytes are counted for each bird and the heterophill to lymphocyte ratio was calculated.

The data collected from different parameters were subjected to one way ANOVA by estimating least square difference method using SPSS statistical software, version 10.0

RESULTS AND DISCUSSION

The humoral immunity, cell mediated immunity and heterophill to lymphocyte ratio was studied against NDV, SRBC and using PHA-P antigen. The results showed that, there was a significant difference ($P<0.05$) in titer levels for the antigens and decreased stress due to zinc supplementation in diets.

Estimation of SRBC titers

The values for immunological parameters are presented in table 1. The values were estimated for SRBC are significant ($P<0.05$) at the end of 6 weeks of age. The titer levels were more in T_4, T_5, T_7 and T_8 at (60 and 80 ppm levels of inclusion of both inorganic and organic zinc in diets). The titer levels (Figure : l)showed an increase in trend with increase in dietary zinc supplementation. The antibody titer for SRBC ranged from 4.63 (T_1) to 6.87 (T_8). The organic zinc supplementation in diets had shown a better immune response compared to inorganic zinc supplementation. The results were similar to the findings of [8, 9, 10] who concluded that, supplementation of diets with zinc were shown to increase progeny antibody titers to SRBC. The findings of [11] who reported that, there was enhanced humoral immunity with dietary zinc supplementation.

Estimation of NDV Titers

The titers against NDV (table 1) were estimated at the end of 6^{th} week of age and the values are statistically significant ($P<0.05$). The titers ranged from 3.88 (T_1) to 6.12 (T_9).

There was an increase in the antibody titer levels in the treatments supplemented with both inorganic Zinc Sulphate and organic zinc methionine; with organic zinc supplemented diet titers being more compared to inorganic. These titers indicate that the birds in the treatments with zinc as dietary supplement have got better immunity over the control group. The results indicated that, organic zinc supplemented diets showed better immune response against NDV (Figure 1) than inorganic zinc supplemented diets. The results were similar to the findings of [9, 10] who concluded that, zinc in diets had shown to improve immune response. The results were also similar to the findings of [12, 13, 14] who indicated that addition of zinc in diets has improved immune response against NDV.

Estimation of CMI Response

The CMI response (table 1) was estimated by injecting PHA-P intra dermally into wattles and the thickness after 24 hours is taken to calculate the percent increase in thickness. There is significant difference between control and treatment groups ($P<0.05$). The treatments T_2, T_4, T_5 and T_7 showed better CMI response compared to control (Figure 1) indicating that, increase in levels of zinc increased the CMI status of the birds. The delayed type of cell mediated immunity was studied by using PHA-P antigen and the results ranged from 32.29 ± 2.06 to 133.21 ± 1.36. Similar findings were observed by Kidd *et al*, (1992a) who concluded that dietary zinc was shown to increase progeny antibody titers to PHA-P. Whereas the findings of [15, 16, 17, 18, 19] revealed that, there was no affect of dietary zinc on immune response of broilers.

Table 1. Effect of zinc supplementation on immune response to SRBC, NDV titers, PHA-P (CMI) and HL ratio

Treatment (Zn in mg/kg)	SRBC titers	NDV titers	CMI Index	HL Ratio
1 (0)	4.63 ± 0.18^{d}	3.88 ± 0.30^{c}	67.72 ± 6.45^{c}	0.64 ± 0.001^{a}
2(20)	5.62 ± 0.18^{cd}	4.88 ± 0.40^{bc}	78.12 ± 2.93^{bc}	0.57 ± 0.03^{ab}
3(40)	5.75 ± 0.36^{cd}	5.25 ± 0.56^{b}	84.22 ± 2.74^{b}	0.58 ± 0.001^{ab}
4(60)	6.25 ± 0.72^{bc}	5.62 ± 0.26^{b}	61.01 ± 7.67^{cd}	0.49 ± 0.008^{c}
5(80)	6 ± 0.65^{bc}	5.88 ± 0.44^{b}	55.24 ± 1.65^{d}	0.49 ± 0.002^{c}
6(20)	5.37 ± 0.26^{cd}	5.38 ± 0.32^{b}	74.40 ± 4.95^{bc}	0.48 ± 0.002^{c}
7(40)	6.5 ± 0.77^{bc}	5.50 ± 0.26^{b}	32.29 ± 2.06^{e}	0.52 ± 0.002^{bc}
8(60)	6.87 ± 0.35^{ac}	5.87 ± 0.30^{b}	65.24 ± 2.87^{cd}	0.47 ± 0.001^{cd}
9(80)	6.37 ± 0.37^{bc}	6.12 ± 0.35^{ab}	133.21 ± 1.36^{a}	0.51 ± 0.003^{bc}
SEM	0.17	0.12	4.52	0.01
SIGNIFICANCE	*	*	*	*

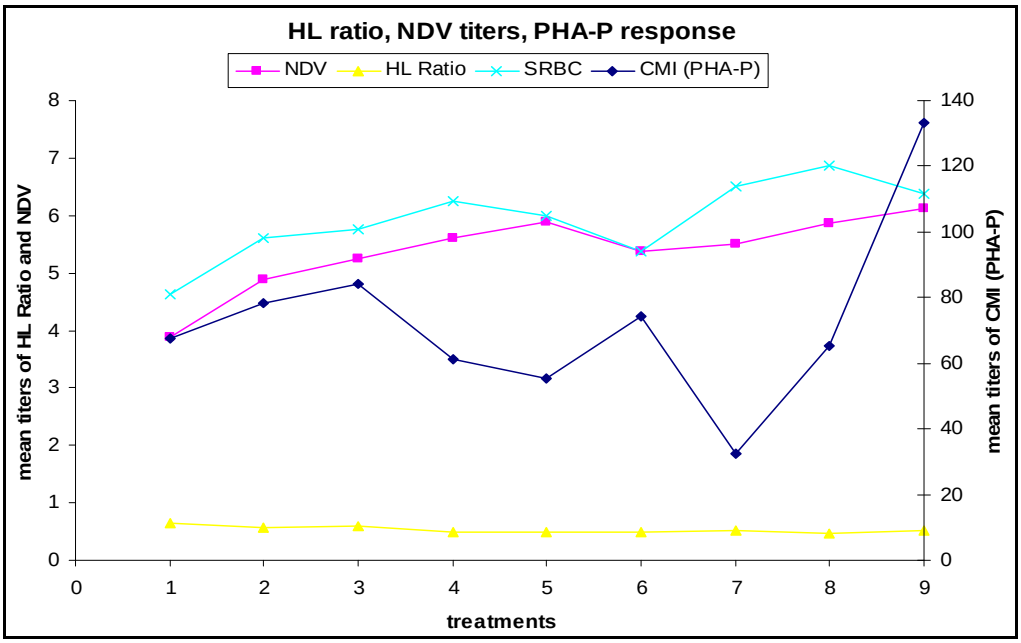
** Significant at (P<0.05)*

Estimation of heterophill to lymphocyte ratio

The heterophill to lymphocyte (H:L) ratio (table 1) was estimated between the various treatment levels. The H:L values ranged between 0.47 (Ts) to 0.64(T_1). There is a significant difference ($P<0.05$) between the ratios of control group and the other treatment groups. The lower ratio indicates that, less stress was experienced by the birds during the experimental period. Among the various treatments the T_4, T_5, T_6, and T_9 experienced less stress compared to control group which indicate that, supplementation of zinc at these levels did not increase stress in the birds. As the values (Figure 1) are below 0.5 it suggests that, there was only less to optimal stress in birds due to dietary zinc supplementation. These results were similar to the findings of [20] who suggested that, the heterophill to lymphocyte ratio of about 0.2, 0.5 and 0.8 are characteristic of low, optimal and high degrees of stress respectively.

The findings are similar to the conclusions drawn by [21] who indicated that H: L ratio was accepted as a reliable and accurate physiological indicator of stress response in chickens and exposure to stress would increase the ratio progressively.

Fig. 1 Effect of dietary supplementation of zinc on immune response



CONCLUSION

The results of the present investigation indicated that zinc supplementation to broiler diets increased immune response. The zinc methionine supplementation was proved better dietary supplement when compared to Zinc Sulphate. The zinc supplementation at 40 mg/kg diet with zinc sulphate and 80 mg/kg diet with Zinc methionine would yield better immune response.

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