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Immunopotentiating action of zinc sulphate in layer chicks

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Zinc Sulphate,
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ELISA
Micro Heamagglutination Inhibition Test
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Abstract

The present study was undertaken to study the immunomodulatory effect of Zinc Sulphate in layer chicks by monitoring the specific immunological responses, to New Castle Disease and Infectious Bursal Disease Vaccines, Three groups of experimental chickens comprising of 20 chicks were taken in which, first two groups of chicks were inoculated twice with Live Newcastle Disease and Infectious Bursal Disease antigens intra occularly. The chicks of group I served as Vaccinated and II group vaccinated with Zinc Sulphate as supplement while group III experimental birds served as Unvaccinated control. At the termination of experiment, the order of seroconversion to inoculated antigens i.e. New Castle Disease and Infectious Bursal Disease were II>I>III and II>I>III while in Total Protein and Globulin levels, Net body gains and Phagocytic Index were II>I>III respectively. Challenge studies were conducted on experimental chicks employing Virulent New castle Disease Virus and Virulent E.Coli (078 serotype) to study the specific and non- specific immune responses respectively. The results indicated better survival/protection rates for Zinc Sulphate treated chicks followed by that of vaccinated group and Unvaccinated chicken groups. It is concluded that Zinc sulphate is more efficient immunomodulator in evoking specific as well as non specific immune responses in layer chicks. So, Zinc Sulphate can be recommended in field use for enhancing the immunological responses besides net body weights in layer chicks along with the Scheduled vaccination programmes.

Introduction

Poultry industry is facing a great setbacks with repeated emergence of various bacterial and viral diseases. Apart from causing mortality in young birds they produce a severe immunosuppression in surviving birds making them more prone to wide range of diseases. The reasons attributed for repeated emergence of disease may lack of quality on the part of vaccine, improper storage and handling of vaccines, lack of proper immunobiological response by vaccinated birds due to stress, inadequate nutritional diets, environmental factors and exposure to various immunosuppressive agents. The variety of stressors either singly or in combination can cause lymphoid involution, increase in Heterophill:lymphocyte ratio [1]and suppress the macrophage function[2]. The antibodies or cell mediated immune response to the infectious agent starts primarily with macrophage activity. Certain viruses and bacteria can replicate in macrophages producing different Cytopathic effects or brings about alteration in cell morphologies associated with macrophage activation. So, the immunomodulating agents in chicken have varying effects on the immune function and have potential to decrease immunosuppression in general[3]. The Zinc Sulphate as dietary supplement in the development and maintenance of immune system is now widely accepted, it consists of antistress activities[4] and enhances mononuclear phagocytic activities[5]. Zinc plays a major role in stimulation of immune system of chickens by increasing the bioavailability of zinc [6]. Zn supplementation in breeder diets has been shown to enhance immunity of their progeny[7,8]. The present study was undertaken to evaluate

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immunobiological response of layer chicks against New Castle Disease and Infectious Bursal Disease vaccines by incorporating Zinc Sulphate as supplement.

Materials and Methods

The present study was carried out for a period of 10 weeks in day old 60 healthy white Leghorn Layer chicks to monitor the serological response against New Castle Disease and Infectious Bursal DiseaseVaccines. Three experimental groups i.e. Group I Vaccinated, Group II Vaccinated and Zinc Sulphatesupplementation and Group IIIUnv accinated Control, each comprising of 20 experimental birds. In groups I and II New Castle Disease vaccine (Lasota strain) were incorporated on 7th and booster on 38th day given Intra occularly at the rate of 0.03ml/bird and later on 60th day of experiment R_2B strain of Newcastle Disease injected at the rate of 0.5ml/ bird intramuscularly. The Infectious Bursal Disease vaccine (Intermediate Plus) given on 14th and booster on 28th day of experiment incorporated Intraoccularly at the rate of 0.03ml/bird. These birds specific immunobiological response was analysed by conducting Micro HI against New Castle Disease[9] and Indirect ELISA against Infectious Bursal Disease[10] at weekly intervals and the nonspecific immune response was depicted at bi weekly intervals by phagocytic index[11] and total protein and globulin levels[12]. The overall growth of birds was analysed by taking the bodyweights at weekly intervals. At last challenge studies, was conducted by taking 10 experimental birds from each group. Each group birds were challenged virulent NDV while remaining 10 birds from each group received virulent *E.coli* culture. Symptoms, pathogenic lesions and mortality patterns were recorded and observed 14 days of post challenge. Assay of Humoral immune response was conducted using Micro Haemagglutination Inhibition test. Reciprocal of highest dilution of the serum where there was complete inhibition of HA was taken as end point. In Indirect ELISA, the plates were kept on ELISA reader to observe the optical densities at 490nm which were converted into ELISA titres by Computer programmes. Total serum proteins, albumins and globulin levels were determined.The absorbance (A) of standard (S) and test(T) were measured against Blank (B) were measured immediately on photocolorimeter with Yellow Green filter and red filter on spectrophotometer at 555nm and 630nm.

Calculations

$$\text{Total Protein gm\%} = \frac{\text{A of (T)}}{\text{A of (S)}} \text{X6}$$

$$\text{Albumin in gm\%} = \frac{\text{A of (T)}}{\text{A of (S)}} \text{X4}$$

Globulins

Globulin concentration was calculated using the formulae Globulin gm% = Total protein in gm% - Albumin in gm%.

The non specific immune response of was assessed by Phagocytic index. Each samples four smears were prepared and stained with Leishman's stain, all four slides were prepared and usually 100 phagocytic cell were counted.

$$\text{Phagocytic index} = \frac{\text{Average no. of organisms ingested per phagocytic cell in test samples}}{\text{Average no. of organism ingested per phagocytic cell in control (unvaccinated group)}}$$

Body Weights were recorded at weekly intervals for 10 weeks and average body weights for each group were calculated. Challenge studies with virulent ND virus was an isolate No.105. The isolate characterized as Velogenic New castle Disease Virus and grouped under C1(Asiatic Type) characterized by Central Veterinary Laboratory (CVL) United Kingdom [13]. The virulent NDV isolate no. 105 was used as the Challenge virus in the present study. The virulent culture of E.coli(MTCC No- 078) Procured from IMTECH Chandigarh, was grown on LB Agar Medium and processed to be given at the rate of 1ml containing 10^9 CFU after challenge, the birds were observed daily for 7 days for development of symptoms and mortality. The pertaining to various parameters were analysed statistically as per the standards methods[14].

Results

The serobiological response against New Castle Disease Vaccine and Infectious Bursal Disease vaccine was monitored by conducting Haemmagglutination Inhibition(HI) and Indirect ELISA at weekly intervals in different groups. The HI titres in Ist and 2nd week shown no significant ($P<0.05$) difference was observed between Groups I & Group II while in group III on 2rd, 3rd 4th and 5th showed decreasing HI titres and it differs significantly ($P<0.01$)from Groups I and II. On 6th week, the booster vaccination has been given at the end of the 5th week and the HI titres increased slightly in both vaccinated groups. On 7th week,

groups I and II were differed significantly at (P<0.01) level. On the 10th week of experiment, the lower HI titres of group I differ significantly at (P<0.01) from Groups II. The IBD ELISA titres also exhibited the similar results with no significant difference upto the 5th week of experiment between Group I and II while Group III which differ significantly (P<0.01) from rest of the groups. Upto the end of the experiment, the statistical analysis, indicated significant difference at (P <0.01) in titre values of group I, II and in III, i.e. Unvaccinated chicks revealed continued presence of maternal bodies to IBD virus. The average body weights of 60 experimental birds in each group revealed no significant (P<0.05) differ within groups upto 3rd week, But in 4th week, the healthy unvaccinated control group showed significantly lower body weights (P<0.01) compared to the vaccinated groups. At the termination of experiment, i.e. on 10th week, ZnSo4 treated group, showed better body weight gains over vaccinated and unvaccinated groups, and differ significantly at (P<0.01). In Challenge studies, 100% survivability was seen in both vaccinated and zinc sulphate treated groups, while 100% mortality was seen in the healthy Unvaccinated control groups. In another, non specific challenge studies, virulent cultures of *E.coli* revealed 70% and 50% of survialibility in zinc sulphate treated and vaccinated group while 100% of mortality was seen in Group III i.e. the unvaccinated control groups.

Table 1: NDV-hi titres* at weekly intervals across groups

Groups	Weeks									
	1	2	3	4	5	6	7	8	9	10
Vaccinated	3.00^{Ae}	4.00^{Ad}	4.00^{Ad}	4.00^{Ad}	4.00^{Ad}	4.667^{Ac}	4.5^{Bc}	5.833^{Ab}	5.833^{Bb}	6.167^{Ba}
Vaccinated + Znso4	3.00^{Ag}	4.00^{Af}	4.167^{Af}	4.33^{Ae}	4.033^{Af}	4.50^{Ad}	5.00^{Ac}	6.00^{Ab}	6.167^{Ab}	6.833^{Aa}
Unvaccinated control	3.00^{Aa}	3.00^{Ba}	2.00^{Bb}	1.717^{c}	0.4^{Bd}	0.00	0.00	0.00	0.00	0.00

*Mean value of Pooled sera samples of 6 birds of each group expressed in log2/0.05ml
Means in the column differ significantly (P<0.01) , Means in the row differ significantly (P<0.01)

Table 2: Efficacy of zinc sulphate on weekly weight gains

Groups	1	2	3	4	5	6	7	8	9	10
Vaccinated	14.005^{Ai}	54.5^{Ah}	142.5^{Ag}	263.75^{Af}	284.5^{Af}	327.0^{Ae}	476.5^{Ad}	530.0^{Ac}	605.75^{Ab}	675.75^{Aa}
Vaccinated+ ZnSo4(supplement)	13.99^{Ai}	48.25^{Ai}	160.0^{Ah}	245.75^{Ag}	278.5^{Af}	317.5^{Ae}	456.25^{Bd}	533.0^{Ac}	620.75^{Bb}	700.75^{Ba}
Healthy Unvaccinated	13.965^{Ai}	46.5^{Ah}	120.75^{Bg}	196.5^{Bf}	216.0^{Be}	229.725^{Be}	407.75^{Cd}	508.5^{Bc}	563.5^{Cb}	630.75^{Ca}

Means in the column differ significantly (P<0.01) ,Means in the row differ significantly (P<0.01)

Table 3: Efficacy of zinc sulphate on ibd-elisa titres at weekly intervals across groups

Groups	1	2	3	4	5	6	7	8	9	10
Vaccinated		5.623^{Ac}	5.679^{Cc}	5.897^{Bc}	7.25^{Aab}	7.13^{Bab}	8.103^{Aab}	9.508^{Aa}	6.365^{Ac}	6.324^{Ac}
Vaccinated+ Znso4		5.695^{Ab}	7.164^{Aa}	7.386^{Aa}	7.805^{Aa}	8.318^{Aa}	8.164^{Aa}	8.745^{Ba}	6.96^{Aa}	6.725^{Ab}
Unvaccinated Control		5.748^{Aa}	7.034^{Ba}	5.078^{Ba}	5.011^{Ca}	5.066^{Ca}	5.07^{Ba}	5.00^{Ca}	5.09^{Ba}	5.101^{Ba}

*Means in the column differ significantly (P<0.01), Means in the row differ significantly (P<0.01)

Table 4: Efficacy of zinc sulphate on total protein levels at biweekly intervals across groups

	Total Protein Levels				
Groups	2	4	6	8	10
Vaccinated	3.563^{Bc}	3.553^{Bc}	5.012^{Ab}	5.098^{Bb}	7.107^{Ba}
Vaccinated+ZnSo4	3.824^{Ad}	3.76^{Ad}	4.963^{Ac}	5.985^{Ab}	7.00^{Ba}
Unvaccinated control	3.313^{Ca}	3.099^{Ca}	3.372^{Ba}	3.358^{Ca}	3.229^{Ca}

Table 5: Efficacy of zinc sulphate on total globulin level across groups

Total Globulin Level					
Groups	2	4	6	8	10
VC	2.068^{Ad}	2.05^{Ad}	2.707^{Ac}	2.919^{Bb}	4.777^{Ba}
VC + ZnSo4	2.102^{Bd}	2.048^{Ad}	2.458^{Bc}	3.432^{Ab}	5.00^{Aa}
HUV	1.839^{Cb}	1.429^{Bc}	2.076^{Ca}	1.82^{Cb}	2.326^{Ca}

Means in the column differ significantly (P<0.01)
Means in the row differ significantly (P<0.01)

Table 6: Efficacy of zinc sulphate on phagocytic index at biweekly intervals across groups

Groups	2	4	6	8	10
VC	1.800	2.100	2.000	4.660	5.000
VC + ZnSo4	2.000	1.900	2.450	5.360	9.000
HUV	1.000	1.000	1.000	1.000	1.000

Means in the column differ significantly (P<0.01)
Means in the row differ significantly (P<0.01)

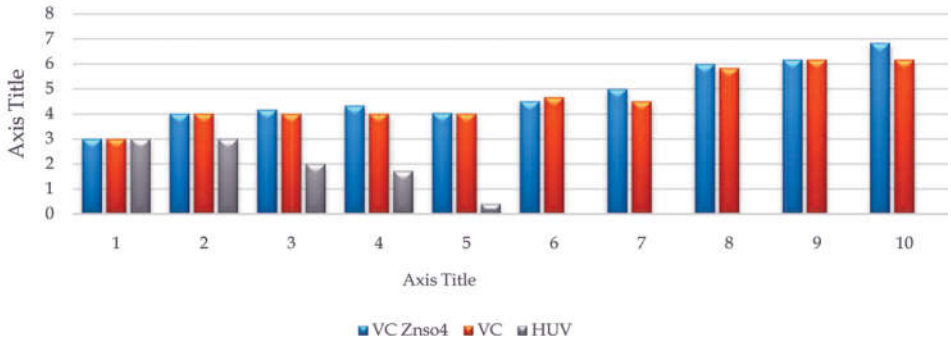

Fig. 1: Ndv-hi titres* at weekly intervals across groups

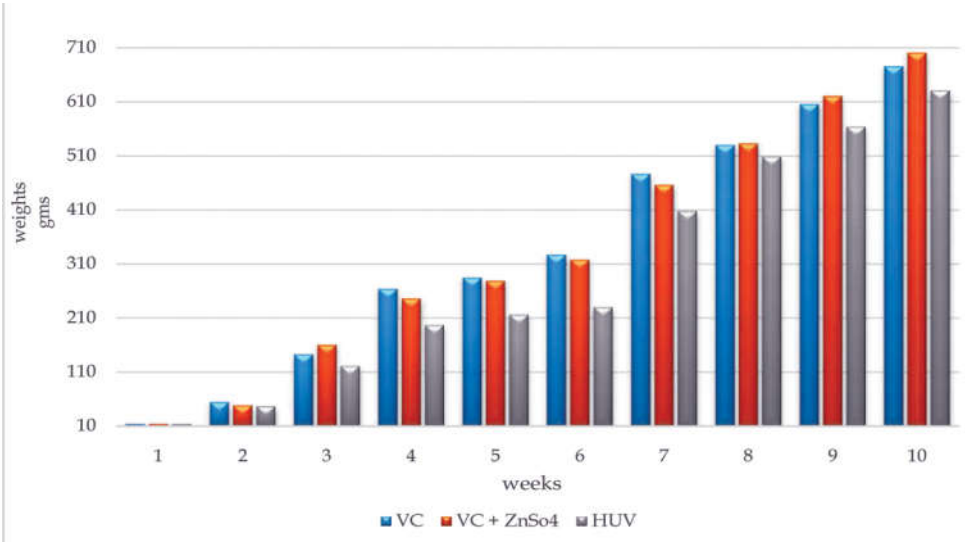

Fig. 2: Comparative efficacy of zinc sulphate on weekly weight gains across groups

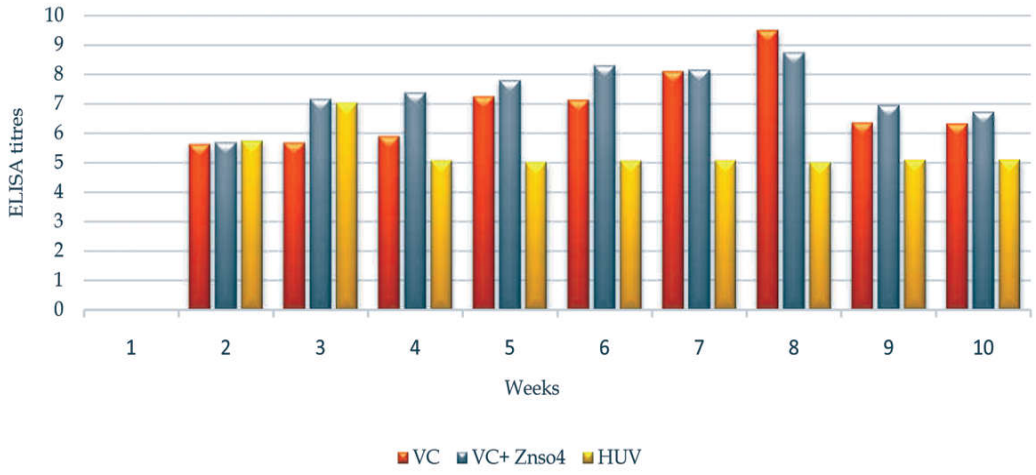

Fig. 3: Efficacy of zinc sulphate ibd-elisa titres across groups

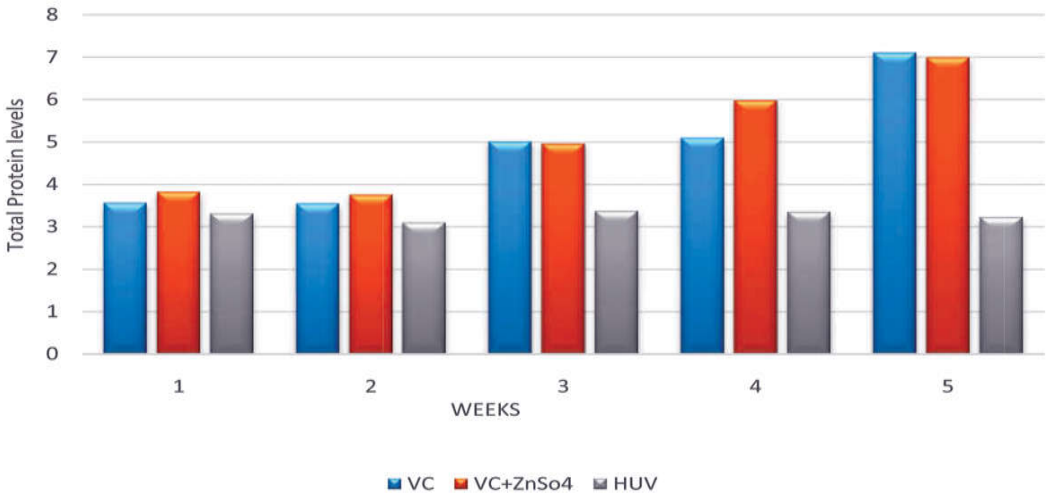

Fig. 4: Comparative efficacy of zinc sulphate on total protein levels across groups

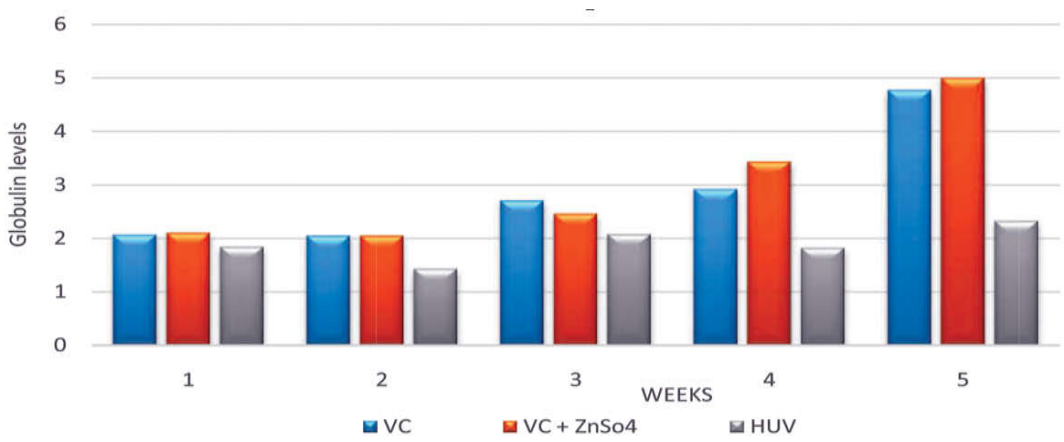

Fig. 5: Comparative efficacy of zinc sulphate on total globulin levels across groups

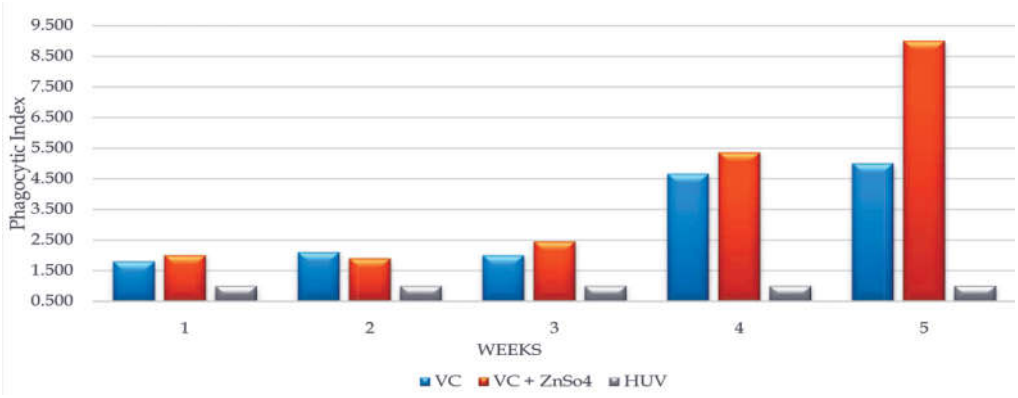

Fig. 6: Comparative efficacy of zinc sulphate on phagocytic index across groups

Discussion

In today's commercial poultry farms, major goal of many poultry producers is to attain good liveability and sustained performance. Most of the poultry feed ingredients usually contains low quantities of pesticide residues, mycotoxins, antibiotics which generally suppresses the immune status of birds, stress, hypoprotaemia, vitamins and mineral deficiency have adverse effects on immune system. Establishment of adequate levels of protective and long lasting immunity to inoculated antigens/ vaccines may require an effective immunopotentiator like adjuvants, liposomes, levamisoles, vitamins like A, E and minerals like Zn, Na, Cl and Se used for this

purpose. Specific immunomodulation implies the increase in both HI and ELISA titres in treated groups compared to untreated groups. Zinc Sulphate treated groups showed increased HI titres against New Castle Disease Vaccine compared to the vaccinated control groups [15],[16]. Their study revealed that Zinc Sulphate enhanced the HI titres significantly in healthy vaccinated and experimentally immunosuppressed birds. It also indicated that Zinc Sulphate supplemented diets improved Cell Mediated Immune as well as antibody response to Pasteurella antigens in healthy mice [17]. Marginal enhancement of ELISA titres due to Infectious Bursal Disease Vaccine were seen in Zinc sulphate treated group however, these were found to be statistically non significant with vaccinated control groups.In young birds, high Maternally Derived Antibodies titres interfere with early vaccination against Infectious Bursal Disease (IBD) using classical modified-live vaccines like Intermediate and intermediate plus and there was very marginal increase of titres [18]. In the present studies, the Zinc Sulphate treated group were not much effective against the live Infectious Bursal Disease Viral vaccinations as Maternally Derived Antibodies interfered with the vaccine and moreover, the studies conducted to know the role of Zinc on immune system in chickens revealed that bioavailability of Zinc in the form of Zinc – methionine resulted in 206% more biologically available when compared to Zinc Sulphate [6]. Supplementation of zinc in the form of chelates of methionine in breeder diet aids in development ofimmune organs and increase in antibody titers to Sheep Red Blood Cells and non-specific immunity in the progeny[10],[17]. The Scientists reported that Zinc deficiency causes hypoplasia of thymus, spleen and other lymphoid organs (bursa), and also decreases T-cell function[19]. More research is needed to throw light in enhancement of IBDV ELISA titres.The total protein and globulin levels in sera were indicated higher protein levels in sera samples which were statistically nonsignificant difference immune response in Zinc Sulphate treated groups to vaccinated groups. The reasons of poor immune response of Zinc Sulphate may be due to less bioavailability of Zinc Sulphate than Zinc methionine which experimentally proved in chicks [6]. Further, it also revealed that Zinc Sulphate was not much effective to reverse immunosuppressive condition in restoring the protein and globulin levels in sera samples of experimental birds[20]. The non immunospecific immune response was assessed by phagocytic index. The phagocytic index in Zinc Sulphate showed higher than vaccinated control groups. It was reported that adequate dietary Zinc supplementation was important for proper functioning of heterophills, mononuclear, phagocytes and T- Lymphocytes which are important for disease resistance. Zinc- deficient mice have impaired killing of intracellular parasites, which is rapidly corrected in vitro by addition of Zinc [21]. Reduced macrophage activity in phagocytosis of Candida sps. was observed in Zinc-deficient animals [22]. Net body gains were also analysed at weekly intervals and results shown that as immnopotentiator has direct action on the growth harmone or due feed conversion efficiency leads increase in the general immune status. The results of challenge test usually gives real indication of success of rate of vaccination or percentage of protection, health and immune status of birds. The resistance to challenge infection in experimental birds indicate combined effect of humoral, Cell mediated Immune response and Nonspecific immunological responses evoked by specific vaccine/antigens and immunopotentiators. In New Disease Virus challenged birds 100% mortality was seen in the Unvaccinated group[23,15]. In *E.coli* challenge studies 70% survivability seen in Zinc Sulphate treated group more than the vaccinated groups. Dietary zinc-methionine enhanced mononuclear phagocytic function against *Salmonella enteritidis* and influences clearance of *E.coli* from blood in young turkeys [24]. The immuno-modulatory role of zinc is mediated through a hormone thymulin which is necessary for lymphocyte development, metalloenzymes (DNA and RNA polymerase) and zinc-dependant deoxythymidine kinase [25, 26]. As, the Zinc supplementation in diets is important against various bacteremia, parasitic infection [27]. Deficiency of zinc leads to decrease the cellular immunity [28] Thymus [29] and spleen [30]. Abnormal T lymphocyte development is thought be the primary consequence of zinc deficiency [25]. Moreover, the Zinc Sulphate treated birds were challenged with the virulent strain of NDV virus 100% survivability was seen while *E.coli* cultures 70% survivability was seen. It was reported that zinc supplementation in breeder diets will enhance the immunity of their progeny [7,8].

Conclusion

The outcome of results on various parameters revealed better performance of Zinc Sulphate as immunomodulator in order to enhance specific and non specific immune response in chickens. Further, more studies are needed to know the appropriate

dosage of supplementation of Zinc salts in the form of chelates to enhance both specific and nonspecific immune response.

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