EFFECT OF CALF THYMUS EXTRACT ON IMMUNITY IN CHICKEN VACCINATED WITH NEWCASTLE DISEASE VIRUS

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by

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CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF CALF THYMUS EXTRACT ON IMMUNITY IN CHICKEN VACCINATED WITH NEWCASTLE DISEASE VIRUS"** *submitted by* **CHANDRANAIK B. M.,** *for the degree of* **MASTER OF VETERINARY SCIENCE** *in* **VETERINARY MICROBIOLOGY,** *of the* **UNIVERSITY OF AGRICULTURAL SCIENCES, DHARWAD,** *is a record of research work done by him during the period of his study in this University, under my guidance and supervision and thesis has not previously formed the basis for award of any degree, diploma, associateship, fellowship or other similar titles.*

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CHANDRANAIK B. M

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ABBREVIATIONS

%	=	Percent
µl	=	Microlitres
@	=	At the rate
ATH	=	Avian thymic hormone
B	=	Bursa derived lymphocyte
BSA	=	Bovine serum albumin
Ca^{++}	=	Calcium ion
cDNA	=	Complementary deoxyribonucleic acid
cm	=	Centimeter
CMIR	=	Cell mediated immune response
Con A	=	Concavalin A
conc.	=	Concentration
CTE	=	Calf thymus extract
$CuSO_4$	=	Copper sulphate
Da	=	Daltons
DHP	=	Dihydro hapato pronol
DLC	=	Differential leukocyte count
DNCB	=	Dinitro chlorobenzene
DTH	=	Delayed type of hypersensitivity
E. coli	=	*Escherichia coli*
ELISA	=	Enzyme linked immunosorbent assay
FTS	=	Facteur Thymique Serique
g	=	Grams
HA	=	Haemagglutination test
HI	=	Haemagglutination Inhibition test
HIR	=	Humoral immune response
hrs	=	Hours
I/N	=	Intra Nasal
I/O	=	Intra ocular

I/P	=	Intra peritoneal
IBDV	=	Infectious bursal disease virus
IL-2	=	Interleukin – 2
iu	=	International units
Log	=	Logarithmic value with base 10
LPS	=	Lipopolysacharride
mg	=	Milligrams
Mg^{++}	=	Magnesium ion
mins	=	Minutes
ml	=	Milliliters
Na_2CO_3	=	Sodium carbonate
NBT	=	Nitroblue tetrazolium
ND	=	New castle disease
NDV	=	New castle disease virus
NH_4Cl	=	Ammonium chloride
NS	=	Normal saline
OD	=	Optical Density
PBL	=	Peripheral blood leukocyte
PBS	=	Phosphate buffer saline
PHA	=	Phyto haemagglutinin
PI	=	Phagocytic index
PMA	=	Phorbol myristate acetate
S/C	=	Sub cutaneous
SE	=	Standard error
Std	=	Standard
T	=	Thymus derived lymphocyte
THF	=	Thymic humoral factor
TLC	=	Total leukocyte count
TVT	=	Transvenereal tumor
WBC	=	White blood cell

Introduction

1. INTRODUCTION

The ever changing path of innovation and modernization has lead to the metamorphosis of primitive poultry farming system into an industry which is highly profitable, contributing substantially to national exchequer and making India one of the top nations in the field of poultry production.

Amidst vast improvement in the field of disease chemo-prophylaxis, the poultry industry still faces sudden disease outbreaks resulting in heavy economic disasters which are attributed to high morbidity, mortality and an increased cost of treatment which usually goes in vain.

Newcastle disease (ND), a viral disease of domestic birds, has been reported from different parts of the world and the disease still exists as an enzootic in several countries including India. In domestic birds, the disease presents a variety of clinical forms ranging from a mild to a fatal fulminating illness, the severity being determined primarily by the strain of the infecting virus and the level of immunity in the host.

Following its description in 1926, several attempts have been made to control the disease and its spread of several such control measures, vaccination has been most successful method. In spite of regular vaccinations, severe outbreaks often occur in areas of intensive poultry production, which is

reasoned mainly to breakdown in immunity. These factors prompted researchers to work with variety of immunomodulators to restore the immunity.

The thymus is an important organ which plays a crucial role in the formation of the lymphoid structures in the prenatal and early postnatal period of life and orchestrating the lymphoid system throughout the life.

The immune system is mainly attributed to the cells like lymphocytes, macrophages and also non-phagocytic accessory cells. Lymphocytes are the main bodies which comprise of thymus derived T-lymphocytes and Bursa of fabricius derived B-lymphocytes in poultry. Scientists have purified and characterized different chemically defined polypeptides in the thymus, which nonspecifically stimulate the immune system (Burstein *et al.*, 1988) and thereby stimulating the overall defense mechanism of the body which is of great importance for prevention of all sorts of immunosuppressive influences.

Various studies with thymus extract have indicated a variety of beneficial effects. The thymus extract of poultry (Murthy and Ragland, 1984) and mammals (Schulof *et al.*, 1984) showed definite and remarkable immunomodulatory effects. The thymic extract was found to increase lysozyme activity, T-lymphocytes, total proteins and haemoglobin in calves (Nikitenko *et al.*, 1984), restored the depressed functions of humoral and cellular immune systems in alloxan diabetic mice (Hadzija *et al.*, 1987). It also resulted in a

partial regression of metastatic hepato-cellular carcinoma (Palmieri *et al.*, 1990). The thymus extract enhanced the blastogenic response of peripheral blood lymphocytes to phytohaemagglutinin (PHA) and concavalin (conA) mitogens in chicken (Murthy and Ragland, 1992). Thymus extract has been found to enhance the antibody titers in birds vaccinated against infectious bronchitis virus, infectious bursal disease virus (Barbour *et al.*, 1998) and NDV (Mohammed *et al.*, 1995).

The immunomodulatory effect of thymus extracts of various species has been attributed to the thymic factors /hormones present in the thymus.

Very little work has been done in India and abroad in this aspect with ND still existing as a major threat to poultry industry either through mortality or through loss of egg production in layer birds. Keeping in view the above immunomodulatory effects of the thymus extract, the present study was taken up with the following objectives.

(1) Preparation of calf thymus extract (CTE) and its partial purification.

(2) Study of the effect of CTE in NDV vaccinated layer chicken on,

   a. Humoral immune response

   b. Cell mediated immune response and

   c. Non specific immune response.

Review of Literature

2. REVIEW OF LITERATURE

2.1. Immunomodulatory role of thymus

Dependence of immune system upon thymic microenvironment is well documented. Experiments involving thymectomy of neonatal mice (Miller, 1961) and rabbits (Archer and Pierce, 1961) revealed that there was atropic lymphoid tissue and increased susceptibility to infections resulting due to retardation in immune potential. It was further demonstrated (Osoba and Milller, 1963) that thymic tissue implanted in thymectomised mice was able to partially restore immunocompetence, suggesting soluble thymic factors as important signals in orchestration of a competent immune response. Subsequently, different chemically defined polypeptide factors which have immunomodulatory activities were isolated from thymus of different species, viz., poultry (Murthy and Ragland, 1984), calf (Burstein *et al.*, 1988) and other mammals (Schulof *et al.*, 1989). The studies have revealed a tremendous immunomodulating and other beneficial effects.

2.1.1 Thymic factors and hormones: The immunomodulatory role of thymus or thymic extract is attributed to an array of similar but distinctly different biologically active polypeptides, produced and secreted by the thymus, many of which have yet to be isolated and characterized. The existence of several thymic factors capable of exerting influence over T-cell development as well as serving as regulatory signals for immune programming have been proved. While

purified preparations are capable of evoking diverse effects, it is unlikely that a single hormone is capable of eliciting a fully competent response from a naive immune system. More plausible, is selective regulation of specific aspects of T-cell differentiation via a battery of modulating agents (Hall, 1993).

2.1.1.1 Thymosin fraction-5, a thymic factor was originally extracted from calf thymus (Hooper *et al.*, 1975). Low *et al.*, (1979) has identified separate polypeptides ranging between 30 and 50, several of which contributed to its functional characteristics.

Cohen *et al.*, (1975), studied enhanced proliferative effect in mixed lymphocyte reactions and Wara and Ammann (1975), observed activation of T - cell rosettes.

2.1.1.2 Thymopoietin, which was initially termed thymin was isolated from bovine thymus (Goldstein, 1974). Two thympoietins 1 and 2 have been isolated, characterized and sequenced based on their amino acid residues. Biological activities of thymopoietin were shown by Goldstein (loe. cit), who recorded the induction of several T-cell alloantignes in normal bone marrow cultures. Further, Kook and Trainin (1974), reported that thymopoeitin induces thymocyte maturation. Sunshine *et al.*, (1978), proved that thymopoeitin enhanced allogenic response in peripheral thymus derived lymphocytes.

2.1.1.3 Thymic humoral factor (THF), was initially studied as a crude cell free extract from bovine thymus tissue (Trainin *et al.*, 1966; Kook *et al.*, 1975). Trainin and Small (1970), purified THF, and Burstein *et al.*, (1988) identified THF γ-2 as the component responsible for much of the functions associated with thymus extract.

Small and Trainin (1967), observed that THF increased antibody forming cells in neonataly thymectomised mice and during 1971 they found that there was an increased immunocompetence in bone marrow culture.

2.1.1.4 Thymulin and thymulin like factors, the most important hormone was initially isolated (Pleu *et al.*, 1977; Bach *et al.*, 1978) as Facteur Thymique Serique (FTS) from porcine serum. It has been purified, sequenced and synthesized with synthetic product, exhibiting activities comparable to the native nanopeptide in available bioassay systems. Thymulin is now recognized as the factor which is responsible for most of the immunomodulatory effects of the thymus extract of various species.

Bach *et al.*, (1975) reported that thymulin increased the induction of thy - 1 antigen expression in thy-1 negative precursor cells. Brand *et al.*, (1977) in their *in vitro* study using thymulin indicated that thymulin increases the lymphocyte differentiation.

Nikitento *et al.,* (1984) reported that thymus homogenate enhanced the natural resistance of calves and piglets with increased lysozyme activity, more T-lymphocyte, more of total blood proteins and more haemoglobin at 2-3 months of age. Chang W. P. and Marsh (1993), studied the effect of the *in vitro* exposure of avian bone marrow cells and thymocytes to synthetic thymulin and found that thymulin enhances peanut agglutinin binding to both bone marrow cells and thymocytes.

Mohammad *et al.,* (1995) found that intra-peritoneal injection of calf thymus extract in chicken vaccinated against New castle disease virus resulted in increased total leucotyic count, increased serum globulins and increased percent of active phagocytes.

Barbour *et al.,* (1998) studied humoral and cell mediated immuno-potentiation in vaccinated chicken layers by thymic hormones. Thymic hormone, thymulin was administered intra-peritoneally at the rate of 0.1 ml per bird, at an interval of three days, for a period of three weeks to the white leghorn chicken layers, starting at 29 weeks of age. Subsequently the NDV vaccine was given at 29 and 31 weeks of age. It was observed that 86.4 % increased humoral immunity and highest cell mediated delayed hypersensitivity reactions.

Abdel-Fattah *et al.*, (1999) showed that oral administration of thymus extract, markedly and significantly increased the total protein, albumin, globulin levels and total leukocyte count and found that chicken administered thymus extract and vaccinated with infectious bursal disease (IBD) vaccine showed 100% protection against challenge with IBDV.

2.1.1.5 Avian thymic hormone (ATH), Pace *et al.*, (1978) identified a thymus specific antigen originally designated T1 within the plasma membrane and cytosol of chicken thymus cells. Avian thymic hormone was capable of inducing physiological changes including increased T-cell marker expression (Murthy *et al.*, 1984), enhanced graft vs. host response of immature bone marrow cells (Vasquez, 1989) and enhanced blastogenic response to T-cell mitogens (Murthy and Ragland, 1992).

Localization by immunoflorescence (Murthy *et al.*, 1984) and immuno-histological staining (Hall *et al.*, 1991) has isolated ATH to cortical reticulo-epithelial cells, primarily near the cartico medullary junction. Murthy and Ragland (1984), studied and characterized several common physico-chemical and biological properties of ATH with several mammalian thymic hormones and factors.

A cDNA library has been prepared and utilized in hybridization studies, confirming ATH as unique to avian thymic tissue (Palmisano and Henzel,

1991). Expression and purification of recombinant ATH was achieved in *E. coli*. The availability of recombinant ATH may facilitate further investigations into functions as well as mechanisms of action and control of this interesting protein.

2.2 Specific immune response

2.2.1 Humoral immune response (HIR)

2.2.1.1 Haemagglutination inhibition (HI) test:

The HI microtest procedure developed by Allan and Gough (1974) was used to assess the haemagglutinating (HA) antibody. With this test a titre of $8(2)^3$ could be assumed to indicate immunity (Spradbrow *et al.*, 1988) although chicken with lower levels of antibody might be protected. The chicks were vaccinated with NDV, through intranasal, intraocular routes and by seven days post inoculation NDV specific antibodies were detected. On intratracheal inoculation, NDV specific antibodies were detected at fourth and seventh day's post inoculation with enzyme linked immunosorbent assay (ELISA) and HI tests respectively (Marqurot, *et al.*, 1985). ELISA and HI test were compared for their ability to measure the primary serological response of chickens inoculated by intranasal, intraocular routes with NDV and secondary response after intra tracheal challenge. Results concluded antibodies against NDV in serological profile studies, the dose or route of inoculation might influence the relative correlations between anti-NDV ELISA and HI test.

Mohammed *et al.*, (1995) used HI test to asses the antibody level in chicken vaccinated and administered calf thymus extract both orally and intra-peritoneal injection. There was not much difference of HI antibody titres (26.8 $\pm$ 6.6) when administered orally but there was an enhancement of HI antibody titres (28.8 $\pm$ 9.9), in birds inoculated with CTE intra-peritoneally when compared to antibody titres of control birds (26.8 $\pm$ 8.6).

Rehmani and Spradbrow, (1995) inoculated the chickens with live V4 strain of NDV by oral route which resulted in the production of circulating antiviral antibodies. There was a low HI antibody response to inactive vaccine which was not enhanced by inoculation of adjuvant.

Singh, (1995) assessed the antibodies against NDV by using HI tests in egg yolks and sera from commercial poultry farms. Literature on monitoring of HI antibodies and NDV vaccinated birds treated with CTE was scanty except the one reported by Mohammed *et al.*, (1995).

2.2.1.2 Serum Proteins

Nikitenko *et al.*, (1984) found that use of thymus homogenate in calves and piglets increased the total blood proteins.

Mohammed *et al.*, (1995) studied the effect of calf thymus extract on immunity in chickens and recorded that treated birds had an increased serum proteins and serum globulins.

Abdel Fattah *et al.*, (1999) showed that administration of crude thymus extract significantly increased the total blood proteins and globulin with marked increase in lymphocyte count

2.2.2 Cell Mediated Immune (CMI) response

2.2.2.1 Di Nitro Chloro Benzene (DNCB) test

It is an established fact that direct application of compounds like DNCB to skin will result in systemic sensitization to various metabolites of the sensitizing compound. The ability of an individual to develop contact sensitivity is a measure of cellular immunity and DNCB has been employed to evaluate the CMI response in man and animals (Brummerstedt and Basse 1973; Bosworth *et al.*, 1975; and Stites, 1978).

The cutaneous sensitization test using chemical compound like 2, 4-dichloro benzene was considered as one of the dependable tests for measuring the cell mediated immunity status and with this objective, the test was successfully carried out in man and animals from time to time (Brummersteat and Basse, 1973; Maisel and Obgura, 1973; and Bosoworth *et al.*, 1975).

DNCB can induce DTH reactions in chickens without adjuvation with molecular weight of 200 Daltons (Da). DNCB itself is unlikely to be an immunogen. It is probably bound to chicken host material to form a hapten-self carrier for the induction of sensitivity. Formation of such a hapten self carrier in guinea pigs and in humans has been shown by Silberg *et al.*, (1974). Takahashi *et al.*, (1977) showed long lasting hypersensitivity reaction in mice after a single painting of DNCB.

It was established that direct application of chemically reactive compound like DNCB to skin resulted in systemic sensitization to various metabolites of sensitizing compounds. These chemical react with skin components to form hapten carrier molecules. DNCB was considered as highly reactive substance which could form dinitrophenyl protein complex with various skin components (Provost, 1978). The ability of an individual to develop contact sensitivity was considered to be a measure of cellular immunity to a new antigen to which it was not exposed previously (Stites, 1978).

Rajan, *et al.*, (1981) standardized DNCB test for evaluating CMI responses in goats and was found to be effective in evaluating cell mediated immunity.

Valsala, *et al.*, (1981) evaluated CMI response where in 2 to 2.5 mm increase in thickness of skin in 24 hrs after injecting the 2-4 DNCB in ducks.

They also studied the DTH reaction and histopathological features of the reaction to the chemical. Tizard, (1982) described that allergic contact dermatitis developed in skin following exposure of tissue cells to reactive chemicals, consequent to formation of protein chemical complexes and their subsequent rejection through immune response.

Narayana Bhat and Iqbal Ahmad, (1988) studied the CMI response in transmissible vaginal tumor (TVT) affected and to clofibrate treated and healthy control dogs by cutaneous sensitivity test using 2-4 DNCB. They found significant difference in skin thickness prior to challenge with DNCB and 24 hrs to 48 hrs after challenge in all the three groups of dogs.

Hari babu *et al.*, (1993) assessed the CMI response in cattle affected with rinderpest using 2-4 DNCB. Karnatak *et al.*, (1993) studied the immunomodulatory effect of levamisole on the antibody response to ND vaccination by using DNCB test to asses CMI. Rao *et al.*, (1995) used DNCB skin sensitivity test to asses CMI response in this study on Zeetress on immune response of chicks vaccinated with IBD Ramadevi *et al.*, (1996) used DNCB for assessing CMI in broiler chickens fed with ocharatoxin A. Pralhad, (2000) used DNCB for assessing CMI response in chicken vaccinated against ND and treated with chicken IL-2.

2.3. Non specific immune response

2.3.1. Nitro blue tetrazolium (NBT) reduction assay

The phagocytic activity of phagocytes can be measured by its capacity to ingest a pale yellow salt of NBT and to reduce it to a dark blue coloured compound. The intensity of NBT reduction roughly correlates with bactericidal activity (Park *et al.*, 1968).

Frymus *et al.*, (1985) estimated serum lysozyme (LZM) activity and NBT reduction capacity of circulating phagocytes in foals and mares, which revealed that circulating phagocytes from foals had similar NBT reduction potency to cells from mares.

Nagahata *et al.*, (1986) assessed bactericidal activity of bovine neutrophils by an improved quantitative reduction assay and intracellular killing assay. Stefaniak (1986) compared the effect of various doses of heparin on the result of NBT in cattle and concluded that phagocytic activity was not affected much by heparin at 3 units/ml. Siwicki and Studnicka, (1987) investigated phagocytic ability of neutrophils and serum lysozyme activity in experimentally infected 'cyprinus capriol' when infected with *Pseudomonas alcaligenes* and *Aeromonas punctata* by NBT reduction rate.

Granatova, (1989) determined the phagocytic activity of phagocytes in pigs vaccinated against Auzesky's disease. Where phagocytes were stimulated either by starch particles or left unstimulated by using tetrazolium reduction

test. Karmanska *et al.*, (1989) used NBT reduction test for the diagnosis of parasitic infection *Trichinella spiralis* in some organs of mice. Silva *et al.*, (1989) studied functional capabilities of morphologically mature (segmented) and immature granulocytes (neutrophils and eosinophils) from bone marrow of cows and compared similar activities of segmented granulocytes from blood, phagocytosis of *E. coli*, and post phagocytic oxidative metabolic stimulation measured by NBT reduction assay.

Yoneyama *et al.*, (1989) studied the effect of Dihydro hapato pronol (DHP) on neutrophil function by using NBT reduction and phagocytic killing of *Staphylococcus aureus* were also studied to confirm the effect of DHP on neutrophil function. Criag *et al.*, (1990) assayed colorimetric microplate assay by using NBT reduction test for the leucotoxin of *Pasteurella haemolytica*. They concluded that inhibition of NBT reduction after stimulation of ruminant leukocytes with phorbol myristate acetate (PMA) can be used as a simple, specific assay for leukotoxin and for antileukotoxin antibodies.

Panchbai *et al.*, (1990) stated that staining of WBC with NBT could not reflect the neutrophil function against autogenous vaccine, where as TVT is a chronic granulomatous disease condition which revealed diminished NBT positive neutrophils.

Ganai and Jha (1991) showed significant reduction in the nonspecific activity of spleenic reticulo-endothelial cells due to *Lantana camara* intoxication by using NBT. Talwar (1992) described NBT slide test for assessment of neutrophil function. Nakagava *et al.*, (1993) found that an active egg white product enhanced non-specific host defense mechanisms against bacterial infection in mice by NBT reduction and intracellular killing of *Staphylococcus aureus* by neutrophils.

Fuente *et al.*, (1993) studied the effect of physical exercise on the phagocyte function of peritoneal macrophage from swiss mice (measuring oxidative metabolism burst) by NBT reducing test. Perrera *et al.*, (1973) evaluated phagocytic process of head and kidney granulocytes of tench (*tinca tinca*-2) with *Candida albicans* as well as latex beads by NBT reduction assay. Krumosych *et al.*, (1996) studied phagocytic activity of neutrophils in horses and concluded that LPS activates phagocytic activity by NBT reduction assay. Pralhad, (2000) evaluated phagocytic process of peripheral leukocytes of chicken vaccinated against ND and treated with avian IL-2 using NBT reduction assay.

Materials and Methods

3. MATERIALS AND METHODS

Following are the materials and methods employed to study the effect of calf thymus extract on immunity in chicken vaccinated against NDV. The present work was conducted in the Department of Veterinary Microbiology, Veterinary College, Bidar.

3.1 Source of birds

A total of 40, day-old layer chicks (BV-300) were obtained from Venkateshwara Hatcheries limited – Hyderabad, for the study.

3.2. Vaccine

The Lentogenic and Mesogenic vaccine of NDV, the Lasota and R_2B strain respectively were used for vaccination. Vaccines were procured from 'Ventri Biologicals', Venkateshwara Hatcheries Limited, Hyderabad.

3.3. Calf thymus extract

Calf thymus extract was prepared as per the method employed by Nikitenko *et al.,* (1984) with slight modifications. Briefly, 100 gms of calf thymus tissues were collected in sterile phosphate buffer saline (PBS) (pH 7.2), washed thoroughly with PBS. It was well triturated and subjected to Teflon homogenization. The extract was collected and diluted 1:10 by PBS. The

prepared samples remained for two hours at room temperature, and then heated at 60°C in a water bath for 30 mins. The same was further boiled for two minutes, passed through whatman filter paper No. 2. The extract was autoclaved for an hour at 120° C. The protein estimation in the thymus extract was done by the method of Lowry *et al.*, (1951) and was adjusted to a conc. of 1.8 mg/ml.

3.4 Protein estimation of thymus extract

The protein concentration of the homogenized calf thymus extract was estimated by Lowry Folin Cio Calteu reaction (Lowry *et al.*, 1951) using the following materials.

3.4.1 Materials:

a. Copper reagent

(i) 4 % Na_2CO_3: 4 g of Na_2CO_3 was dissolved in 100 ml of double distilled water.

(ii) 4 % sodium potassium tartarate: 4 g of sodium potassium tartarate was dissolved in 100 ml of double distilled water.

(iii) 2 % $CuSO_4$: 2 g $CuSO_4$ was dissolved in 100 ml of double distilled water.

All the above reagents viz. i, ii, iii, were mixed in proportion of 100:1:1 respectively and stored at 4°C.

b. Folin and Ciocalteu phenol reagent.

c. Bovine serum albumin (BSA) (Sigma) was used as standard protein and prepared by adding 100 mg of BSA to 100 ml of distilled water.

d. Normal saline (NS) (0.85%) used as a blank.

e. Photoelectric colorimeter.

3.4.2. Method:

a) Calf thymus extract of 0.1 ml was taken

b) BSA of 0.1 ml was used as standard.

c) The volume was made to 1 ml with NS

d) One ml of NS was taken as blank

e) To each tube 5 ml of copper reagent was added, mixed well and kept for 10 mins.

f) 1 ml of diluted working Folin's reagent was added and mixed well.

The tubes were kept in dark for 30 mins and colour intensity was read at 640 nm in a photoelectric colorimeter.

The standard curve was drawn for different amount of BSA standard solution. Using standard curve, the concentration of unknown protein was calculated depending on the optical density (OD) of respective solution.

3.5 Treatment protocol

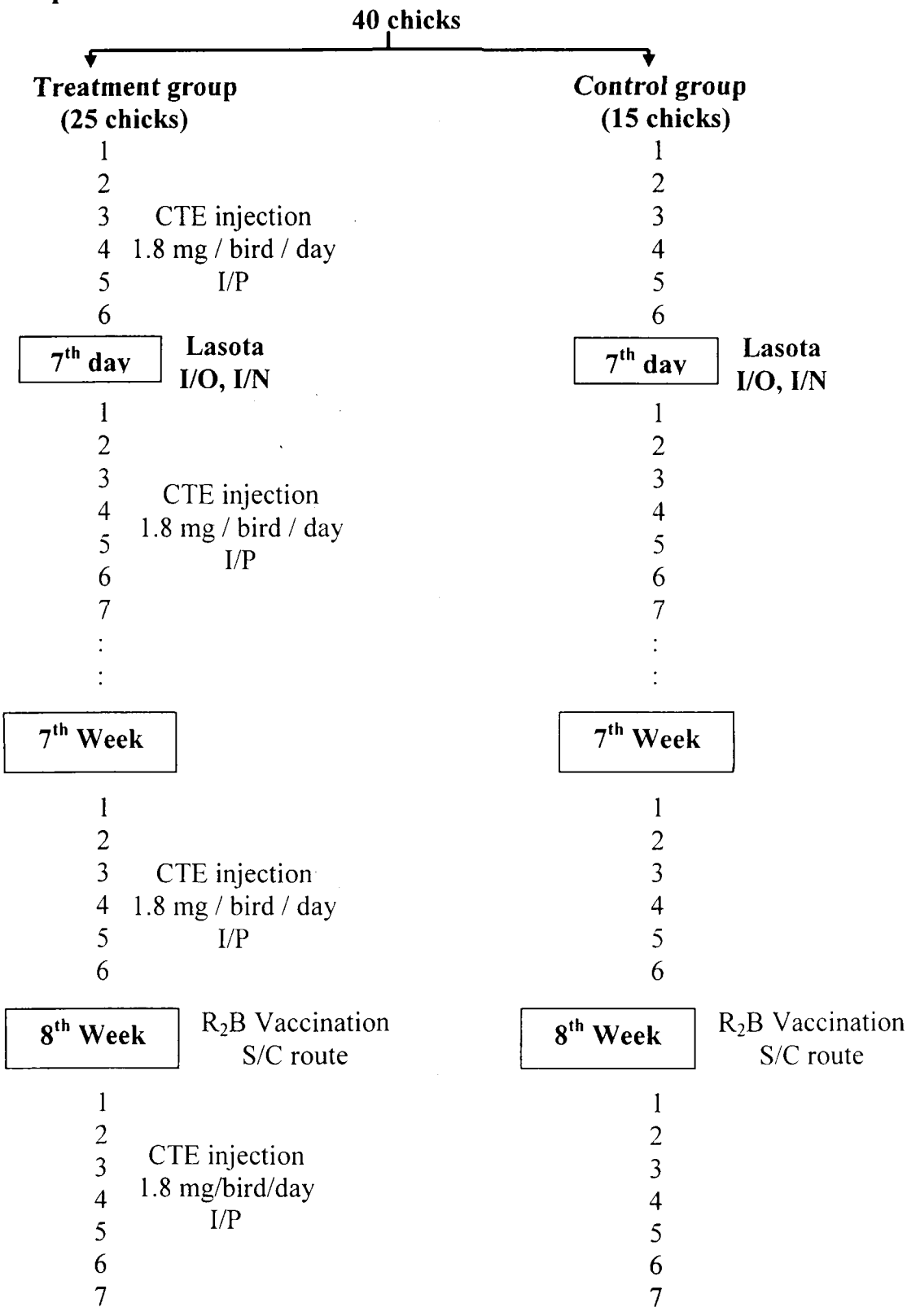
3.5.1 Vaccination schedule

A total of 40, day-old commercial layer chicks were divided into two groups, first group comprising of 25 chicks, which was the test group and the second with 15 chicks as the control group. Both groups were vaccinated with Lasota strain of NDV on 7^{th} day by I/O and I/N routes. Mesogenic strain of NDV (R_2B) on 8^{th} week by subcutaneous route.

3.5.2 Treatment with CTE

The chicks in the test group were administered with CTE @ 1.8 mg/chick/day, by Intra peritoneal (I/P) route for one week prior and one week after each vaccination. The control group remained as just vaccinated group, without administration of CTE.

3.5.3 Experimental flow chart



Note : Samples were collected on the 15th day after each vaccination

3.6 Sampling from birds

3.6.1 Blood

Heparinised blood (20 IU of heparin per one ml of blood) was collected from wing vein from each bird after 15th day of each vaccination from both the test as well as control groups. About 10 to 15 ml of blood was collected from each group for isolation of peripheral blood lymphocytes and smear preparation for differential leukocytic count (DLC) and for conducting total leukocyte count (TLC).

Also 10 – 15 ml of blood was collected without anticoagulant for serum separation from both the groups.

3.6.2 Isolation of peripheral Blood lymphocytes (PBL)

Lymphocytes were separated according to the method of Boyum (1968) by density gradient centrifugation with some modifications.

The heparinized blood was collected from wing vein and was centrifuged at 1000 g for 30 mins. The buffy coat was collected and diluted to four volumes in Ca^{++} and Mg^{++} free Dulbecco's PBS (DPBS) (pH 7.2). Then the cell suspension was layered on to Histopaque, the proportion of cell suspension to Histopaque was adjusted to 3:1. Centrifugation was carried out at 400 g for 30 mins. The interface ring containing mononuclear cells was collected and treated

with 0.83 % Ammonium chloride (NH_4Cl) solution to lyse erythrocytes if any. The cells were washed in DPBS to remove platelets completely.

3.7 Haemagglutination Inhibition (HI) test

3.7.1. Materials:

a. Normal saline (0.85%)

b. 0.8 % chicken erythrocyte suspension.

c. Microplates

d. Diluters and droppers.

e. Serum samples

f. NDV antigen – 4 HA units.

3.7.2 Method:

The micro-test method described by Allan and Gough, (1974) was used for detection of H1 titers from serum samples collected on 15th day post immunization of birds. The HI test was done manually by β-procedure in 'U' bottom micro-plates using diluters, droppers and 4 HA units of ND viral antigen.

Serial two fold dilution of serum in normal saline is taken and 25 µl/well 4 HA unit of antigen is added. Plates were incubated for 45 mins at room temperature. 50 µl of 0.8 % erythrocytes were added to each well and the plates were incubated for one hr at room temperature before reading results. The titres

were expressed as the reciprocal of highest dilution of serum showing the haemagglutination inhibition or button formation.

3.8 Serum Proteins

Protein estimation in the serum collected from birds was done using modified Biuret and Dumas method (Dumas, 1971) using diagnostic reagent kit for *in vitro* determination of total proteins and Albumin in serum, supplied by span diagnostics limited, Surat, India.

3.8.1 Total Proteins

3.8.1.1 Principle

Proteins in serum react with copper of Biuret reagent in alkaline medium to form a blue purple complex with maximum absorption at 550 nm.

3.8.1.2 Materials

1. Serum
2. protein standard
3. Modified Biuret reagent
4. Colorimeter

3.8.1.3 Method

	Blank (B)	Standard (S)	Test (T)
Serum	-	-	0.1 ml
Protein Standard	-	0.1 ml	-
Modified Biuret reagent	5.0 ml	5.0 ml	5.0 ml

Mix well and allow the tubes to stand at room temperature for 5 minutes. Measure the O.D of the standard and test in a colorimeter at 550 nm against blank.

3.8.1.4 Calculations

Serum total proteins (gms/100 ml) =

$$X = \frac{\text{O.D of test}}{\text{O.D of standard.}} \times \text{concentration of proteins in standard}$$

3.8.2 Albumin

3.8.2.1 Principle

Albumin in serum binds with the dye bromocresol green at pH 3.68 to form a green coloured complex, the absorbance of which is measured at 600 nm.

3.8.2.2 Materials

1. Serum
2. buffered dye reagent
3. Protein standard
4. Colorimeter

3.8.2.3 Method

	Blank (B)	Standard (S)	Test (T)
Serum	-	-	0.03 ml
Protein Standard	-	0.03 ml	-
Buffered dye reagent	4.5 ml	4.5 ml	4.5 ml

Mix well and allow the tubes to stand at room temperature for 5 minutes. Measure the O.D of the standard and test in a colorimeter at 550 nm against blank.

3.8.2.4. Calculations

Serum total proteins (gms/100ml) =

$$Y = \frac{\text{O.D of test}}{\text{O.D of standard.}} \times \text{concentration of proteins in standard}$$

3.8.3. Calculations for globulins

Serum Albumin (gms/100 ml) =

Z = serum total proteins –serum albumins

i.e. Z = X –Y

3.9 Assay of CMI using 2, 4-Dinitrochlorobenze (DNCB)

Cutaneous hypersensitivity test was performed as per Haribabu *et al.*, (1993).

3.9.1 Materials

a. 2 % DNCB in acetone

b. Vernier's calipers

3.9.2 Method

After each vaccination on 15th day DNCB test was performed. Ten birds from test and five birds from control group were taken. The area of 3 cm diameter skin on neck region was chosen and the hair clipped off close to the skin. 0.05 ml of 2 % DNCB in acetone was applied slowly drop by drop on to the marked area. The solution was made to evaporate quickly by gently blowing and thereby preventing the solution to run down the neck region. The thickness of the skin before challenge (0 hrs) and at 24 hrs and 48 hrs was taken

by using vernier calipers. The comparative difference between the CMI response elicited in test group and control group was evaluated.

3.10. Assessment of phagocytic index by Nitroblue Tetrazolium (NBT) reduction assay

The phagocytic activity of the phagocytes can be measured by its ability to ingest a pale yellow salt of NBT and to reduce it to a dark blue colored compound. The intensity of NBT reduction roughly correlates with bactericidal activity. Phagocytic index (PI) was assessed by NBT reduction assay as per method described by Park *et al.*, (1968) with slight modifications.

3.10.1 Material

a) PBS (0.5 M), pH (7.2)

b) leukocyte suspension : 1 x 10^7 / ml in PBS

c) NBT (0.2% inPBS)

d) NDV antigen

Activated plasma was prepared by mixing 0.15 µl of NDV antigen to 1 ml of normal human plasma.

e) 0.8% aqueous safranin

3.10.1.1. Reaction mixture used

a)	Leukocyte suspension	0.4 ml
b)	Activated plasma	0.1 ml
c)	NBT solution	0.8 ml

Reaction mixture was incubated at 37° C in water bath with shaker for 30 mins and reaction was stopped with cold PBS. Then it was centrifuged at 1000 rpm for 5 min. Supernatant was discarded and a drop of PBS was added and gently the cells were resuspended.

A drop of this reaction mixture was put on a clean slide and allowed to spread, it was dried and fixed in methanol for 2 mins. Stained with 0.8 % aqueous safranin for 2 min. Then the smear was washed, dried and mounted in PBX mounting media. NBT positive cells were counted under oil immersion of microscope.

3.11 Total leukocyte count (TLC)

TLC in both groups was carried out from blood samples collected on 15th day post vaccination. The procedure followed was as per the method of Nambiar, (1960).

3.12 Differential Leukocyte count (DLC)

DLC was carried out as per Nambiar, (1960) using blood collected on 15th post vaccination day, for both control and test groups.

Results

4. RESULTS

Immunomodulatory effect of calf thymus extract (CTE) in chicken vaccinated against NDV was assessed by various assays to study humoral, cell mediated and non-specific immune response.

4.1 Preparation and partial purification of CTE

It was done as per the method explained by Nikitenko *et al.*, (1984) with slight modifications. Thymus weighing about 100 gms yielded 630 mg of thymic protein on protein estimation. It was adjusted to contain 1.8 mg/ml.

4.2. Haemagglutination – Inhibition (HI) titres

The serum samples collected from birds on 15^{th} day after each vaccination were subjected to HI test, the results are shown in Table – 1 and Fig. 1.

The mean log HI titres after primary vaccination with 'F' strain on 7^{th} day were 2.705 ± 0.05 in the treatment group, which received both vaccination and CTE administration. The titres in control group were 2.003 ± 0.06 which were only vaccinated but not administered with CTE.

After second vaccination with mesogenic strain of NDV the titer values were in increasing trend in treatment group with 2.955 ± 0.39. The titer values

remained almost same in control group as in primary vaccination with mean log titer values of 2.053 $\pm$ 0.08.

The results in the present study indicated that there was significant increase of NDV antibody titres in treatment group ($P \leq 0.01$) when compared to control groups.

4.3. Serum proteins

The serum protein concentration in birds after each vaccination is presented in Table – 2 and Fig. 2 and 3.

In control group, the total protein concentration was 3.15 $\pm$ 0.69 and 3.18 $\pm$ 0.16 gms percent after first and second vaccinations respectively. In the test group the total protein concentration increased significantly ($P \leq 0.01$) in relation to control with values of 5.36 $\pm$ 0.26 and 5.95 $\pm$ 0.298 gms percent respectively.

There was significant rise ($P \leq 0.01$) in serum globulin concentration in treated groups after first and second vaccinations with 3.77 $\pm$ 0.255 and 4.22 $\pm$ 0.286 gms percent respectively, while control birds had only 1.93 $\pm$ 0.054 and 1.97 $\pm$ 0.21 after first and second vaccinations respectively. However the albumin concentration did not vary significantly between test and control groups of birds.

4.4 Dinitro chlorobenzene (DNCB) test

DNCB test was conducted on the birds for studying cutaneous type of hypersensitivity, a good indicator of cell mediated immune response, on 15^{th} day after each vaccination.

Twenty four hours after challenge, the area where DNCB was applied, was warm, hyperemic and diffusely oedematous and thickened, the surface presented a granulated appearance due to swelling of the feather follicles.

The results of the DNCB test are given in table – 3 and Fig. 4 & 5. The values are given in millimeters, thickness of the skin read at 0, 24 and 48 hrs after treatment of DNCB on to the skin.

At '0' hours, the skin thickness of birds both in test and control groups remained almost same during both first and second vaccinations with measurements varying between 0.162 ± 0.057 to 0.165 ± 0.019 mm. At 24 hrs the test group had 0.269 ± 0.035 mm skin thickness which was significant ($P \leq 0.05$) when compared to control group with 0.208 ± 0.057 mm of skin thickness after first vaccination. Similarly the test group had a significant ($P \leq 0.05$) values of 0.347 ± 0.039 mm compared to control groups with skin thickness of 0.214 ± 0.41 mm. At 48 hrs the test group showed skin thickness of 0.308 ± 0.038 against $0.236 \pm 0\ 034$ in control groups after first vaccination which was highly significant ($P \leq 0.05$) in relation to control birds.

The maximum reaction of DNCB was obtained in test group after second vaccination with the skin thickness of 0.381 $\pm$ 0.054 mm at 48 hrs which was significant ($P \leq 0.05$) when compared to control birds with readings of 0.256 $\pm$ 0.034, thus indicated that CTE has a profound effect on cell mediated immunity in chickens.

4.5 Nitro blue tetrazolium (NBT) reduction assay

Leukocytes were separated from blood samples, its concentration was calculated and the suspension was adjusted to a value of 1 x 10^7 in PBS. Immediately the suspension was kept in the reaction mixture and the NBT positive cells were identified by calculating those phagocytes in which the cytoplasm colour turned bluish. Whereas in negative cells there was no change in colourations of cytoplasm.

The results of groupwise phagocytic index (PI) of the birds after each vaccination is shown in table 4 and Fig. 6 and 7.

The PI after first vaccination showed levels of 56.4 $\pm$ 6.2 % in treatment group, when compared to control group which showed 24.5 $\pm$ 3.1 %.

The PI values in second vaccination remained same as in first vaccination with 56.2 $\pm$ 8.4 % PI in CTE treated group while 25.1 $\pm$ 7.3 % in control group.

4.6 Total leukocyte count (TLC)

The results of TLC in treatment group and control group on 15th day of each vaccination are presented in table 5 and Fig. 8.

TLC in control group both in the first and second vaccinations were 23.7 $\pm$ 2.98 and 25.6 $\pm$ 3.3 millions/cu. mm respectively, while there was substantial increase in the TLC in test group which was 32.8 $\pm$ 6.12 and 37.3 $\pm$ 8.74 millions/cu. mm respectively, differing significantly ($P \leq 0.01$) in relation to control birds.

The mean TLC values showed that there was significant ($P \leq 0.01$) increase in the total leukocytes in treatment group compared to control group after each vaccination.

4.7 Differential Leukocyte Count (DLC)

The DLC values of both test and control group of birds after each vaccination has been depicted in the table 6 the control group had lymphocyte counts of 57.17 and 58.4 per cent during first and second vaccinations while the test group had an increased mean lymphocyte count of 62.39 % and 65% during first and second vaccinations respectively.

Table – 1: HI Antibody titres after each vaccination

	Control	*Test*
I vaccination (F)	2.003 ± 0.06	2.705* ± 0.05
II vaccination (R_2B)	2.053 ± 0.08	2.935* ± 0.39

Note:

Values are mean of $\log_{10}$ of HI titres values ± SE

* Indicate significantly differ at P≤0.01 compared to control

Figure 1 HI Antibody titres after each vaccination

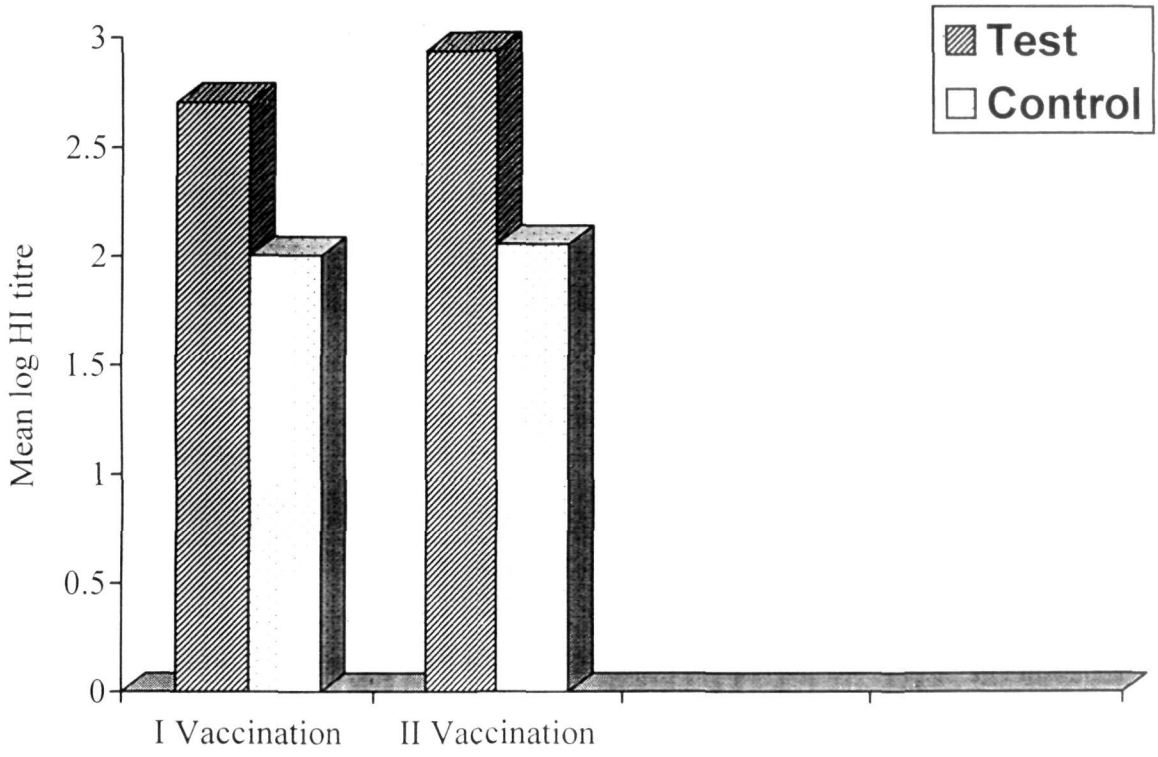


Table – 2: Serum protein concentration in birds after each vaccination

	I Vaccination		II Vaccination	
Parameter in (gm %)	**Control**	**Test**	**Control**	**Test**
Total Proteins	$3.15^{a} \pm 0.069$	$5.36^{b} \pm 0.26$	$3.18^{a} \pm 0.016$	$5.85^{b} \pm 0.298$
Albumin	1.34 ± 0.133	1.58 ± 0.018	1.29 ± 0.102	1.63 ± 0.057
Globulin	$1.97^{a} \pm 0.054$	$3.77^{b} \pm 0.255$	$1.95^{a} \pm 0.21$	$4.22^{b} \pm 0.286$

Note:

Values are mean $\pm$ SE

Those with same superscripts do not differ significantly at $P \leq 0.01$

Figure 2 Serum protein concentration of birds after each vaccination

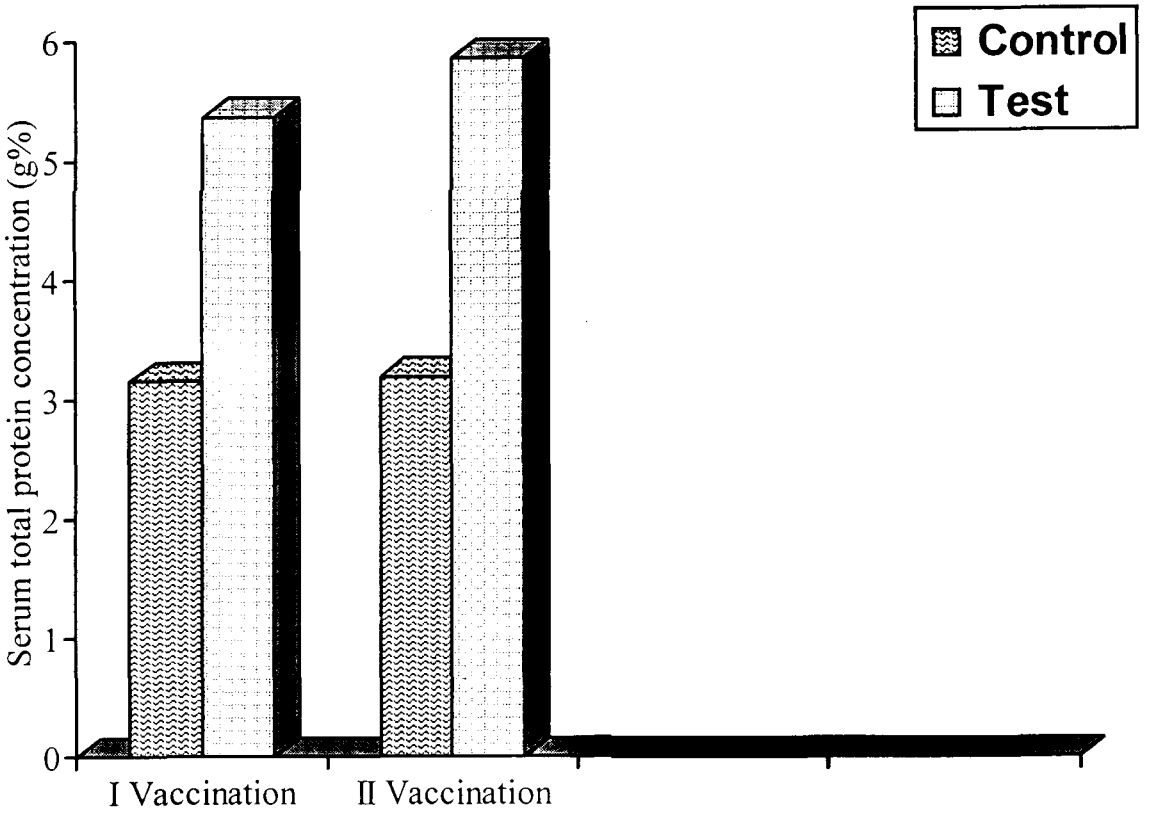


Figure 3 Serum globulin concentration of birds after each vaccination

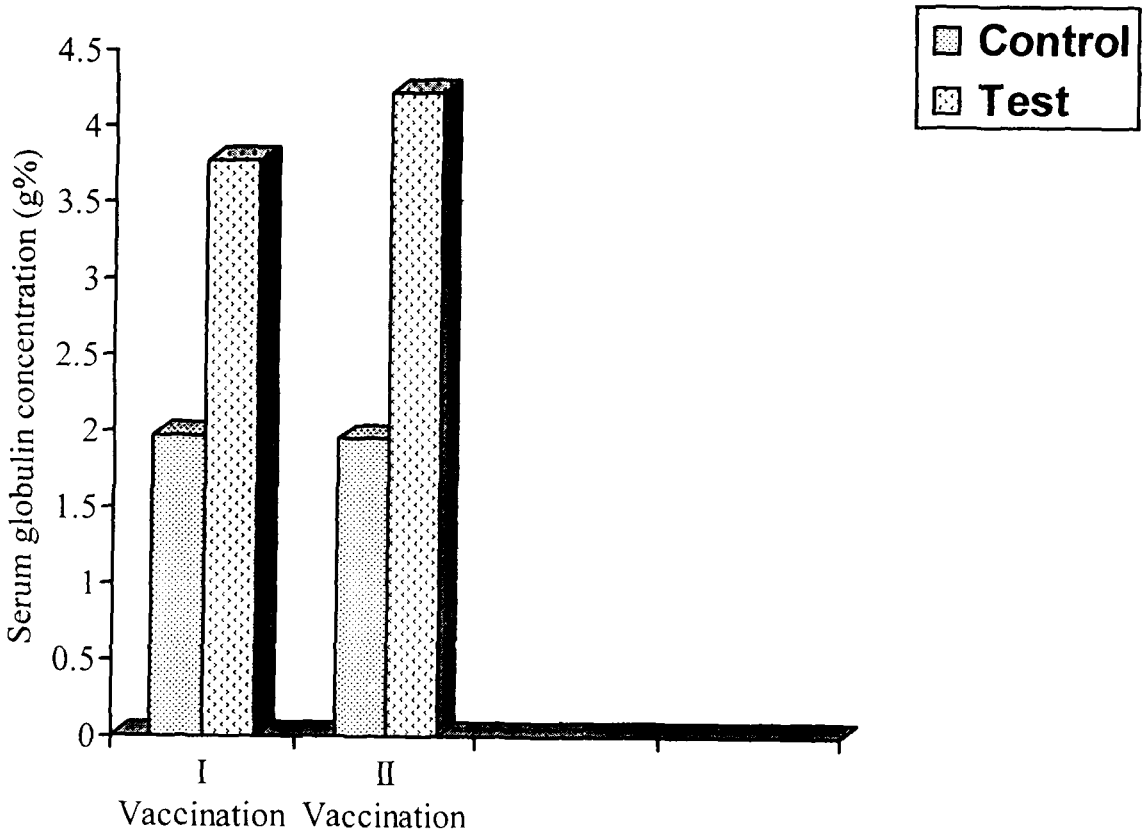


Table – 3: Mean skin thickness (mm) of birds with DNCB test after each vaccination

Time of measurement	I vaccination		II vaccination	
	Control	Test	Control	Test
0 hour	0.163 ± 0.012	0.162 ± 0.031	0.165 ± 0.019	0.165 ± 0.047
24 hour	0.208^{a} ± 0.057	0.269^{b} ± 0.035	0.214^{a} ± 0.41	0.347^{c} ± 0.039
48 hour	0.236^{a} ± 0.034	0.308^{b} ± 0.038	0.256^{a} ± 0.034	0.381^{c} ± 0.054

Note:

Values are mean ± SE

Those with same superscripts do not differ significantly at $P \leq 0.01$

Figure 4 Mean skin thickness (mm) of Birds with DNCB test after each vaccination.

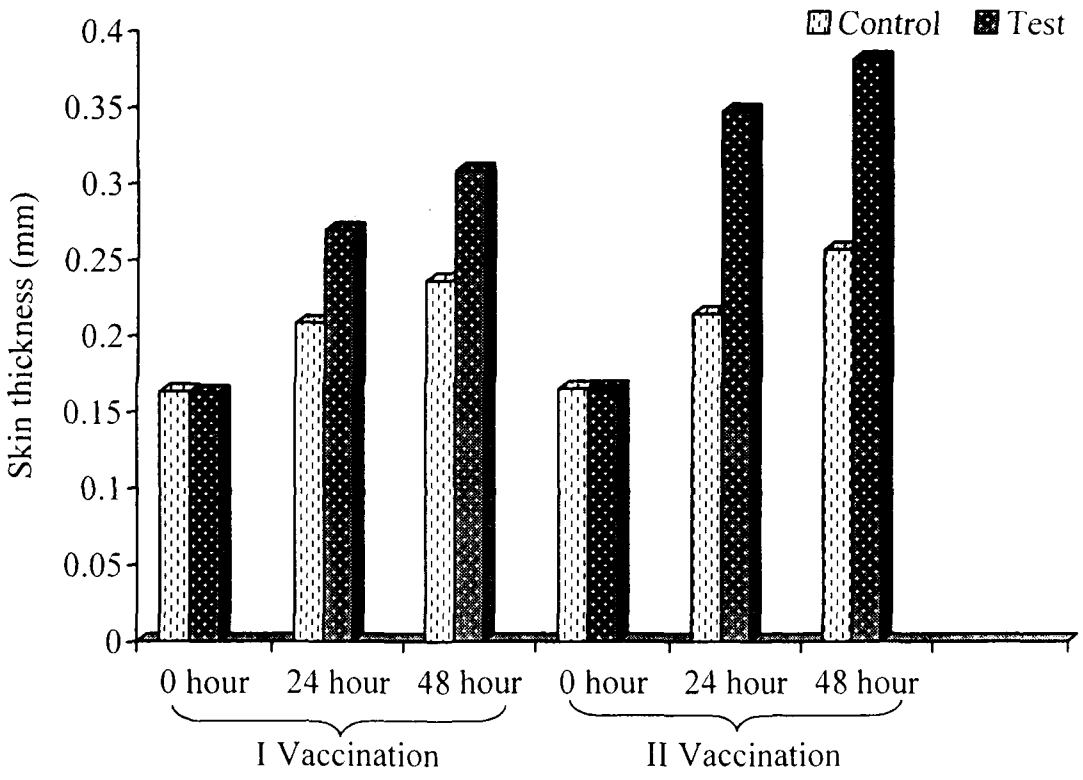


Figure 5. Diffusely oedematous and granular appearance of skin after DNCB sensitization

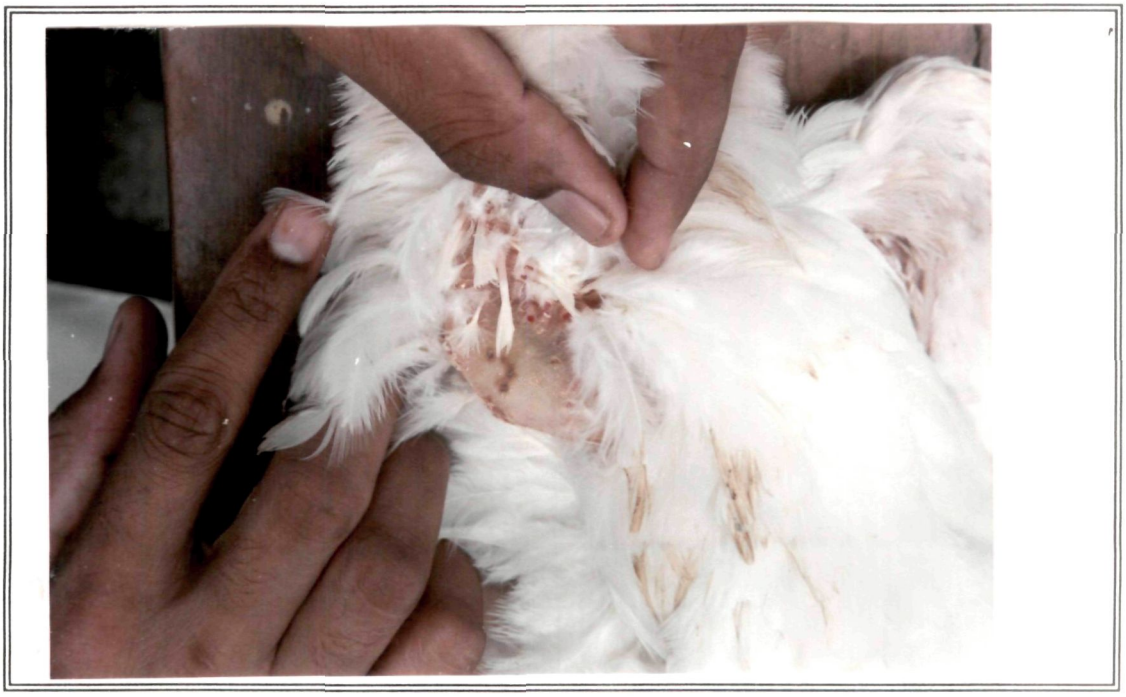


Figure 6. Nitroblue tetrazolium – positive and negative leukocytes

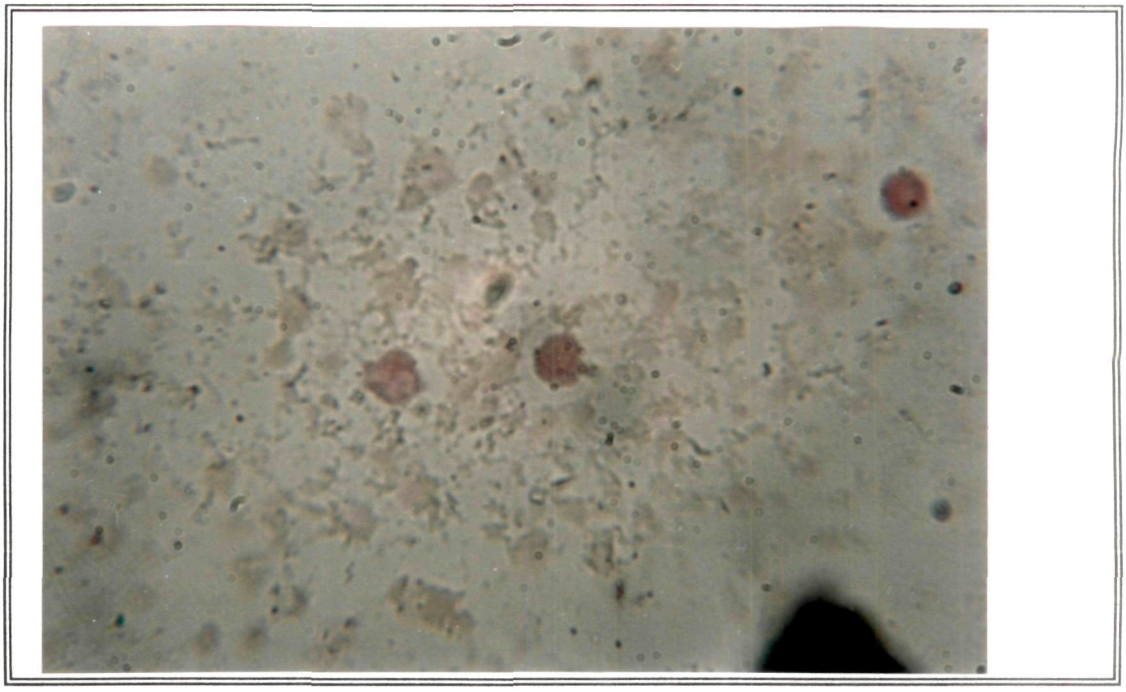


Table – 4: Percent phagocytic index of birds after each vaccination

Percent Phagocytic activity	Control	Test
I vaccination	24.5 ± 3.1	56.4 ± 6.2
II vaccination	25.1 ± 7.3	56.2 ± 8.4

Note:

Values are mean ± SE

Figure 7 Percentage of phagocytic index of birds after each vaccination

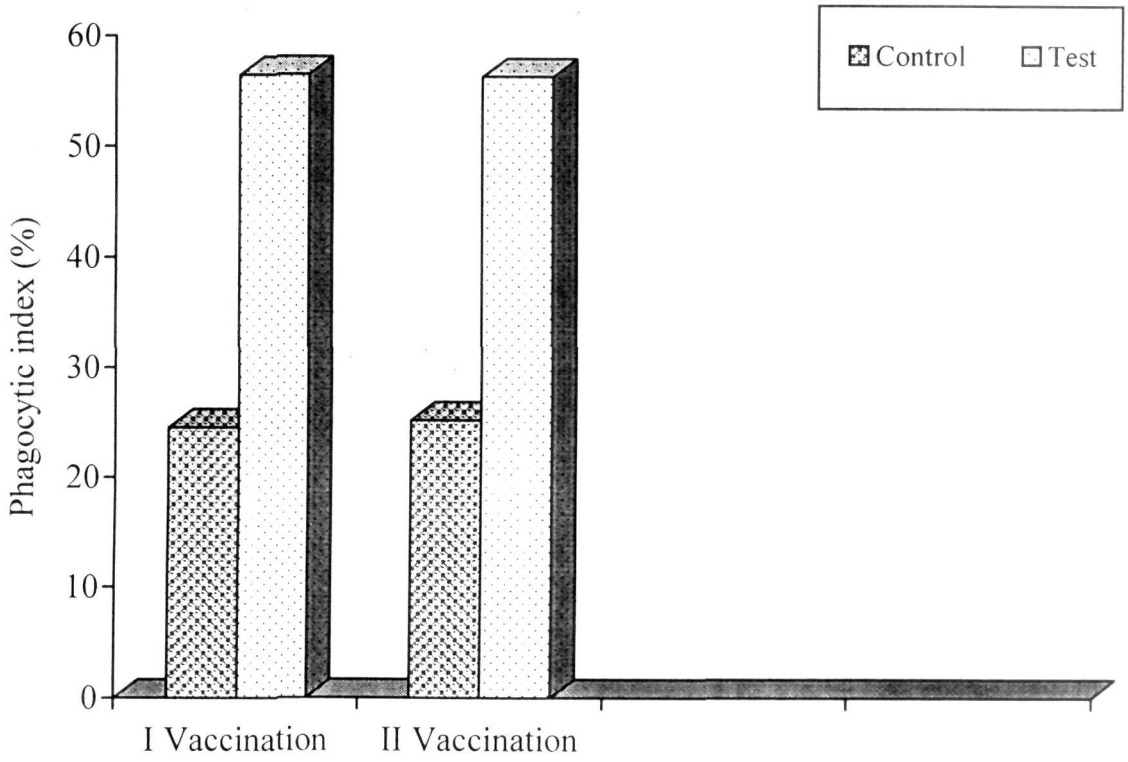


Table – 5: Total Leukocyte count (TLC) in birds after each vaccination

Group	TLC in million/cu mm	
	Control	Test
I vaccination	23.7 ± 2.98	32.8* ± 612.33
II vaccination	25.6 ± 3.33	37.3* ± 8.74

Note:

Values are mean ± SE

* Indicate values significantly differ at P≤0.01 compared to control

The normal value of TLC in birds is 12 to 30 million/cu mm

(Nemi C. Jain, 1986)

Figure 8 TLC in birds after each vaccination

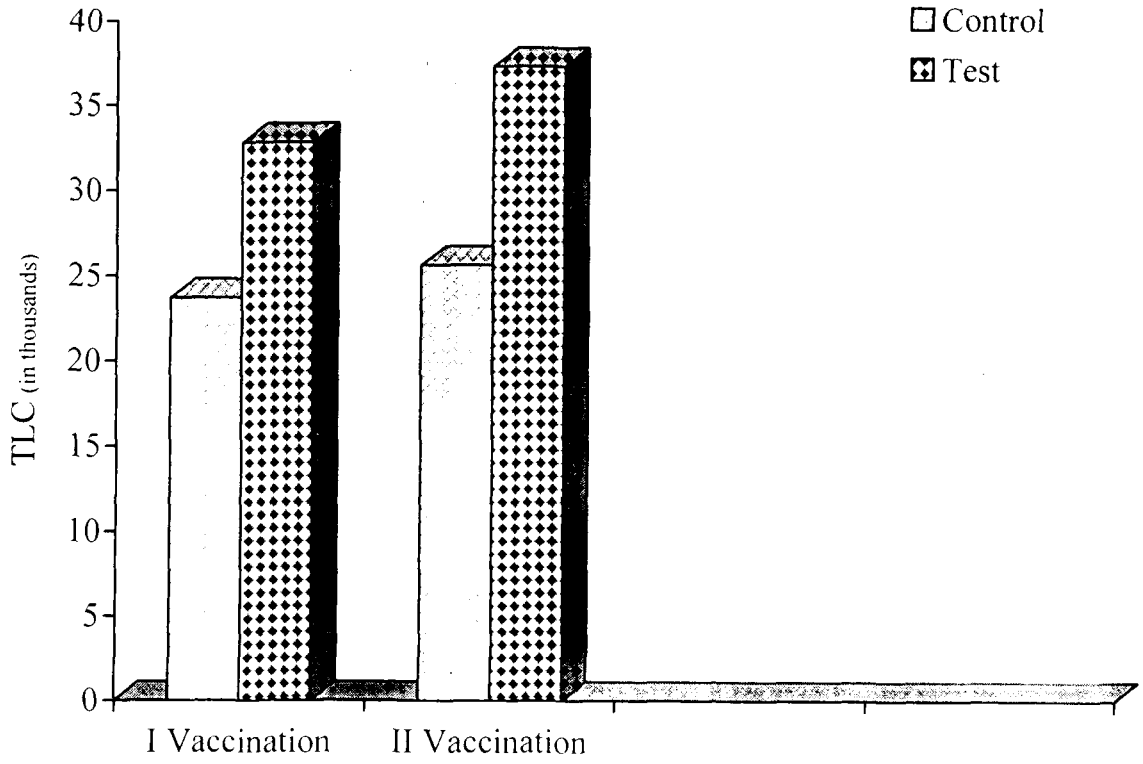


Table – 6 :- Differential leukocyte count (DLC) in birds after each vaccination

Type of WBC (%)	I Vaccination		II Vaccination	
	Control	**Test**	**Control**	**Test**
Lymphocyte	**57.17 ± 2.39 (45-60)**	62.39 ± 3.33	58.4 ± 3.21	65.00 ± 1.2
Monocyte	**6.91 ± 1.23 (5-10)**	6.31 ± 4.0	5.47 ± 1.39	5.11 ± 1.01
Heterophils	**30.84 ± 3.2 (15-40)**	26.53 ± 1.09	31.13 ± 2.4	25.21 ± 2.3
Eosinophils	**1.72 ± 0.72 (1.5-6.0)**	3.22 ± 0.5	2.21 ± 0.46	3.69 ± 0.74
Basophils	**1.03 ± 0.25 (rare)**	0.81 ± 0.63	0.84 ± 0.77	0.73 ± 0.92

Note:

* Values are mean ± SE

The number in parenthesis indicates normal values

Discussion

5. DISCUSSION

In the orthodox scheme of T-cell ontogeny in mammals and birds migrant stem cells colonize the thymus and differentiate to become cortical thymocytes that give rise to medullary thymocytes, and these further differentiate into peripheral T-cells (Weissman, 1973). Thus, in order to fulfill the potential of the thymus as a contributing component of the immune system, it must recruit or be colonized by intrinsic stem cells [Le Douarin, N. M, and Jotereau, F. V. (1986)]. This process of colonization by precursor cells is an important prerequisite for intra thymic T-cell differentiation.

The existence of several thymic factors capable of exerting influence over T-cell development as well as serving as regulatory signals for immune programming have been proved (Hall, 1993).

Immunomodulation, particularly the stimulation of immune response with biologically active substances is gaining tremendous importance with distinguishable promises. The immunomodulation can augment the nonspecific immune response against a wide variety of pathogens and tumors, even in the absence of specific antigen. Again when inoculated with antigen, immunomodulation can behave as adjuvant to augment the specific immune response (Pralhad, 2000).

The present investigation was aimed to asses the adjuvant or immunomodulatory effect of calf thymus extract in chicken vaccinated against NDV.

The protein concentration of CTE extracted in the laboratory was adjusted to contain 1.8 mg/ml as it was the dose given to each chick on the days of treatment with CTE. The dose of the CTE was in accordance with Mohammed *et al.*, (1995).

Nikitenko *et al.*, (1984) used thymus homogenate at the dose of 0.05 ml/kg body weight in calves and piglets injected subcutaneously. Abdel Fattah *et al.*, (1999) used thymus extract @ 0.05 g/chick administering it orally through drinking water.

The CTE was administered by Intra peritoneal route in the present study was as per the suggestions made by Mohammed *et al.*, (1995) who has shown that CTE has a minimum immunomodulatory effect when administered orally or subcutaneously but when injected intra peritoneally, CTE has a profound immunomodulatory effect, thus the dose and route of administration was adopted as per Mohammed *et al.*, (1995) as 1.8 mg/ml/bird, intra peritoneally.

HI titres showed higher humoral immunopotentiation to NDV in the CTE administered group than in the control groups. The work on monitoring of

immune elevels in birds against NDV treated with CTE is very much scanty, however with the available literature, these results were in accordance with results of Mohammed *et al.,* (1995) and Barbour *et al.,* (1998), who recorded highest humoral immunopotentiation to NDV using calf thymus extract and thymulin hormone respectively.

The mechanism of this enhanced humoral immunity could be due to the effect of the immunopotentiators, administered intra peritoneally, on thymic cells, thus raising the thymus dependent humoral antibody responses (Okamoto *et al.,* 1993). It has been well established that thymic hormones like thymulin in the thymus extracts, stimulates lymphocyte differentiation (Brand *et al.,* 1977) , and enhances proliferative effect in mixed lymphocyte reactions (Cohen *et al.,* 1975), probably resulting in increased antibody production, thus enhancing the HI titer values in the CTE treated group.

The serum samples showed an increased level of total proteins in the CTE treated groups compared to the control group, which may be due to the stimulation of thyroid hormones by the crude thymus extract resulting in physiological hyperthyroidism (Abdel Fattah *et al.,* 1999). The increased total serum proteins enhanced the general health status of the birds contributing significantly to the immunity of the birds and also markedly without any mortality of the birds during the entire experiment period. Our results were

consistent with Niketenko *et al.*, (1984), who found that thymus homogenate increases total blood proteins in calves and piglets.

The test group had relatively high concentration of serum globulins over the control groups, the globulin concentration increased in the test group after second vaccination which is also one of the most probable reasons for increase in the HI antibody titres against NDV and results were concurrent with Mohammed *et al.*, (1995) and Abdel Fattah *et al.*, (1999) who have recorded a significant increase in total proteins, particularly the serum globulins in thymus extract administered chickens.

The chicken inoculated with NDV vaccine either alone or with CTE administration were subjected to assess the CMI response. DNCB reaction has been used to assess the delayed type of hypersensitivity by several workers (Valsala, *et al.*, 1981; Hari babu, 1993; and Pralhad, 2000) who have done on different species of animals and birds for various bacterial and viral antigens.

The reactions after DNCB application after 24 hours were similar to the findings of Valsala *et al.*, (1981) wherein the area was warm, hyperaemic, diffusely oedematous and thickened. The surface presented a granular appearance due to swelling of feather follicles. The biometry of skin thickness was maximum in treated group, 0.381 mm, while it was 0.256 mm in control group. The results were in accordance with the findings of Barbour *et al.*,

(1998), who recorded highest immunopotentiation of CMI response in the form of delayed hypersensitivity reaction indicated by the thickness of the wattle after intradermal injection of a trivalent antigen in chickens treated with thymic hormones.

This significant cell mediated immunopotentiation could be due to the ability of the thymus extract to induce the expression of variety of T-cell differentiation markers and to enhance T-cell functional activities *in vivo* and *in vitro* (Bach *et al.*, 1975), to increase mature T-lymphocytes (Parent *et al.*, 1994) and to function as an immunomodulator by exerting control on cytokine production by peripheral blood mononuclear cells (Safieh Garabedian *et al.*, 1993) and the ability to induce lymphocyte maturation (Kook and Trainin, 1974) and to increase lymphocyte differentiation (Brand *et al.*, 1977).

The results of phagocytic index (PI) which was assessed by NBT reduction assay showed non specific immune response to the NDV vaccination. Stimulated peripheral blood mononuclear cells ingest the NBT dye into phagocytic vacuoles and convert it to an insoluble precipitate (Formason) by highly reactive oxygen components. NBT is mainly reduced by the superoxide anion, therefore NBT reduction assay is used as a marker of inra-cellular bactericidal activity of neutrophils and mono nuclear phagocytes.

In CTE treated groups, there was considerable reactivity of NBT (56.4%) compared to control groups (25.1%), which indicated the maximum level of non specific immune response in treated group.

There was no available literature on NBT reduction assay on birds administered with CTE, however there were reports which have indicated the effect of NBT assay as a measure of nonspecific immunity. Park *et al.*, (1968) stated that intensity of NBT reduction roughly correlated with bactericidal activity. The results obtained for the phagocytic activity in CTE treated groups were in accordance with Mohammed *et al.*, (1995) who has shown a significant phagocytic activity in CTE treated chicken.

The increase in phagocytic activity of phagocytes in CTE treated group could be due to the ability of the thymus extract to act as an immunomodulator by exerting control on cytokine production by peripheral blood mononuclear cells. (Safieh Garabedian *et al.*, 1993).

The CTE treated birds, showed a significant increase in total leukocytic count over the control group, which was in accordance with many workers (Mohammed *et al.*, 1995; Barbour *et al.*, 1998; and Abdel Fattah *et al.*, 1999) who have found that thymus extract and thymic hormones increases the total leukocytic count in chicken.

There was a very significant increase in number of lymphocytes in CTE treated groups, when compared to the control group, which is one of the probable reason for over all immunopotentiation and this could be due to the fact that thymic hormones activates T-cell rosettes (Wara and Ammann, 1975) and they enhance the differentiation, maturation and proliferation of lymphocytes.

Nikitenko *et al.*, (1984) found that S/C injection of thymus homogenate increased lysozyme activity and T-lymphocytes in calves and piglets.

The immunomodulation produced by administration of CTE could be attributed to its nucleoproteins accumulated in its cells. The several thymic factors including thymic hormone are probably or at least partially, responsible for thymus immunomodulating activity. The effect of thymus administration appears more obvious in cases of primary or secondary immunodeficiency.

The present study has formed as an unique application part of CTE for assessing the immunomodulatory effect in chicken vaccinated with NDV and treatment has resulted in consistent immunopotentiation of the humoral antibody response, cell mediated delayed hypersensitivity reaction; further more CTE treatment enhanced the non-specific immune performance.

Summary

6. SUMMARY

The present study was made to asses the immunomodulatory effect of calf thymus extract (CTE) in chicken vaccinated against New castle disease virus (NDV) with the objective of preparation and purification of calf thymus extract (CTE) and to study the effect of CTE on humoral, cell mediated and non specific immune responses in NDV vaccinated chicken.

A total of 40, day-old layer chicks were divided into two groups; treatment (test) group with 25 chicks and control groups with 15 chicks. Both the groups were vaccinated with F strain of NDV by I/ocular and I/nasal routes on the 7^{th} day whereas R_2B strain of NDV by subcutaneous route in the 8^{th} week. The chicks in treatment groups were administered with CTE @ 1.8 mg/chick/day by I/P route for one week prior and one week after each vaccination, while the other group remained just as vaccinated control group without CTE administration. Blood samples were collected from birds on the 15^{th} day after each vaccination, from both the groups.

The humoral immune response was assessed using HI titres and serum globulin concentration. The HI test showed higher antibody titres (mean log HI titer of 2.935 ± 0.05) in test group in comparison with control groups, (mean log HI titer of 2.053 ± 0.08). The serum showed highest globulin levels of 4.22 gms % in test group compared to the control which had a maximum of 1.97 gms %, the CMI response was studied using DNCB test by delayed hypersensitivity

reaction after 15th day of each vaccination, in both the groups. The results revealed that cellular reaction was intense in test group (0.381 mm) when compared to control group (0.256 mm).

Phagocytic activity using nitro blue tetrazolium reduction assay was used for studying the non specific immune response. Leucocytes were separated and subjected to NBT reduction assay, test groups revealed highest PI (56.4%) as compared to control group (25.1%). The results indicating CTE enhanced the non specific immune response.

Chickens administered with CTE had an increased total leukocyte count (TLC) (37.3 million/ cu. mm), when compared to values form control group (25.6 million/ cu. mm) and also there was increase in number of lymphocytes in test group compared to control group.

On the basis that the preparations of calf thymus extract are safe to practice in animals and birds, have no allergic, teratogenic, embryotoxic and mutagenic effects (Pridybailo *et al.*, 1989), the present study concludes that administration of calf thymus extract to chicks stimulates the immune system, acts as a growth factor and enhances vaccination effectiveness and the present study might be one of the most significant and valuable contribution for the health of poultry which is always under the threat of diseases and breakdown of immunity.

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7. REFERENCES

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EFFECT OF CALF THYMUS EXTRACT ON IMMUNITY IN CHICKEN VACCINATED WITH NEWCASTLE DISEASE VIRUS

ABSTRACT
2002

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A total of 40, day-old layer chicks (BV-300) were divided into test group (25 chicks) and control group (15 chicks). Both the groups were vaccinated with "F' strain of New castle disease virus (NDV) on seventh day by intraocular, intranasal route and with R_2B strain of NDV in eight week by subcutaneous route.

In test group, calf thymus extract (CTE) was administered @ 1.8 mg/chick/day by intraperitoneal route one week prior and one week after each vaccination where as the control group remained as just vaccinated birds without CTE administration. Blood samples were collected from the birds after 15 days of each vaccination.

The result of haemagglutination inhibition (HI) test showed higher titres (2.935 ± 0.39) in test group in comparison to control group (2.053 ± 0.08). The test group showed significantly higher total proteins (5.85 g %) and globulins (4.22 g %) in the serum compared to control group (3.18 & 1.95 g %

respectively). On application of Dinitrochlorobenzene (DNCB) the test group revealed an intense cellular reaction compared to control group.

The result of phagocytic index (PI) which was assessed by Nitroblue tetrazolium (NBT) reduction assay revealed a higher nonspecific immune response to NDV vaccination in calf thymus administered chicks than in control birds. The blood showed a significant enhancement in the number of total leukocytes, 37.3 $\pm$ 8.4 millions/cu mm in test group, where as it was 2.56 million / cu mm in control birds. There was an increase in percent lymphocytes in test group (65 %) compared to control group (58.4 %).

The overall result indicated that chicken administered with CTE showed higher protective levels of humoral, cell mediated and nonspecific immune response. The result thus advocated that CTE may be used as immunomodulator for enhancing the immunity chicken vaccinated with NDV.