

Original Research Article

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## Age Related Changes in T Cell Subsets in Thymus and Spleen of Layer Chicken (*Gallus domesticus*)

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### ABSTRACT

#### Keywords

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Flowcytometric analysis of T lymphocyte subset *viz.*, CD and CD8 cells in spleen and thymus was done in Nandanam layer chicken of different age groups ranging from day-old to forty weeks. In spleen, the mean CD4 and CD8 population was found to be the highest at day one and lowest at twenty weeks of age. However, an increase in both the T cell population was detected at forty weeks. Among the two cell types studied, CD8 cells were always higher than the CD4 cells, with significant difference ( $P < 0.05$ ) at day one. The distribution pattern of CD4 and CD8 cells in thymus was slightly different from that of spleen. Thymus had more CD4 cells on day one followed with twelve weeks of age. In contrast, CD8 cells were more on twelve weeks followed with day one. The CD4 and CD8 cell populations were lowest in four weeks of age. A distinct difference was observed in the ratio of CD4 and CD8 cells in the spleen and thymus at different age. At day one the ratio was less in spleen and high in the thymus. However, at eight weeks of age, CD4 cell ratio was high in spleen and low in thymus. Nevertheless, the ratio narrowed down at forty weeks of age.

### Introduction

Similar to mammalian species, chicken T-cell populations can be delineated into subsets based on their expression of cell surface proteins. T-cell subsets are broadly defined by the expression of molecules such as CD4, CD8 and T-cell receptor (Erf *et al.*, 1998). Major differentiation events regarding T-cell development in the thymus appear to be conserved between chickens and mammalian

species. Mature CD4 or CD8 single-positive T-cells leave the thymus, populate secondary immune organs, and travel in the circulatory and lymphatic systems. Most CD4+ cells are helper T-cells responding to exogenous antigen in association with major histocompatibility complex (MHC) class II molecules; whereas, CD8+ cells respond to endogenous antigen in association with MHC

class I molecules and generally function as cytotoxic T-cells (Gobel, 1996).

Thymus is the central lymphoid organ occurs in all vertebrate species, situated on either side of the neck, close to the jugular vein (King, 1983). Bone marrow-derived T-cell precursors undergo differentiation, maturation eventually leading to migration of positively selected thymocytes to the peripheral lymphoid organs such as the spleen (Savino and Dardenne, 2000 and Varga *et al.*, 2009) and GALT including the caecal tonsil and the lymph nodes (Ciriaco *et al.*, 2003; Gail Pearse, 2006 and Karen Staines *et al.*, 2013).

Spleen is the principal organ of systemic immunity and its importance in disease resistance is accentuated by the scarcity of avian lymph nodes. The avian spleen functions as a major blood filtering organ and is the major source of antibody production. It does not function as a reservoir of blood as in mammals and its function is not oriented towards supply of oxygen (Jeurissen, 1991). The spleen also plays an important role in erythrocyte destruction, phagocytosis and antigen-antibody interactions (Burke and Simon, 1970).

Due to genetic and environmental differences, avian immune developmental and functional aspects established for layers may differ from broilers. Considering their economic importance, a better understanding of the immune system in broiler chickens is essential.

Nandanam chicken is a dual purpose, colored variety with good disease resistance and most popular among poultry farmers due to its adaptability to backyard farming. Nandanam strain was developed in Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Chennai. Hence, the present study was to find out the relative

proportions of CD4 and CD8 cell populations present in the thymus and spleen of different age groups of Nandanam chicken by using flow cytometry.

## **Materials and Methods**

Materials for this study were collected from six different age groups of Nandanam layer chickens such as day-old, four, eight, twelve, twenty and forty weeks (n=6; each group). All birds were maintained in a healthy environment at Institute of Poultry Production management, TANUVAS, Chennai. The birds appeared healthy and did not show any clinical signs of disease. The birds were euthanized by high cervical dislocation and thymic lobes and spleen were removed for phenotypic analysis of lymphocytes.

To evaluate the lymphocyte populations, single cell suspensions were prepared by finely mincing the thymic and splenic tissue and filter it through 60 micron filters (Wu *et al.*, 2000) and the cell concentration was adjusted to  $1.5 \times 10^6$  cells/ml in RPMI-1640 medium. Then the cells were stained by a single colour immunostaining procedure using a panel of monoclonal antibodies (mAb) as per Chan *et al.* (1988). The mAb panel included mouse anti-chicken CD4: FITC (Cat. No. Serotec MICA 2164F) and mouse antichicken CD8a: FITC (Cat. No. Serotec MCA-2166F) that recognize the avian CD4 and CD8, respectively.

The stained cells were then read in the FACS caliber Flow cytometer (Becton and Dickinson, USA). The lymphocytes were gated based on the FSC and SSC characteristics and using the PBS control cells. The background fluorescence was set as M1 and the fluorescence was set as M2. Percentage of M2 values for different monoclonal antibodies was recorded from the single parameter histogram. The ratio of CD4

and CD8 was calculated by dividing percentage of CD4 cells by percentage of CD8 cells.

Statistical analysis was carried out using statistical analysis software (Systat Inc., Evanston, IL). Test results were considered significant of  $P < 0.01$ . All data were expressed as the mean  $\pm$  S.E.

### Results and Discussion

In the present study by flow cytometric analysis, the CD4 and CD8 count was recorded in the normal spleen and thymus of different age groups of layer chicken (Figure-1 and 2). In spleen, the mean CD4 and CD8 population was found to be the highest at day one and lowest at twenty weeks of age.

Fig.1 Mean CD4 and CD8 T cells in Spleen of different age groups

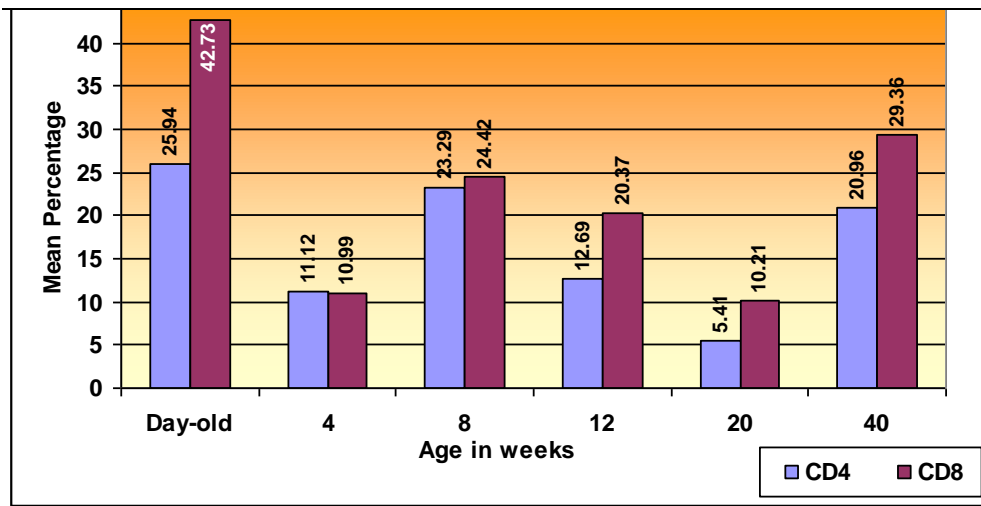


Fig.2 Mean CD4 and CD8 T cells in Thymus of different age groups

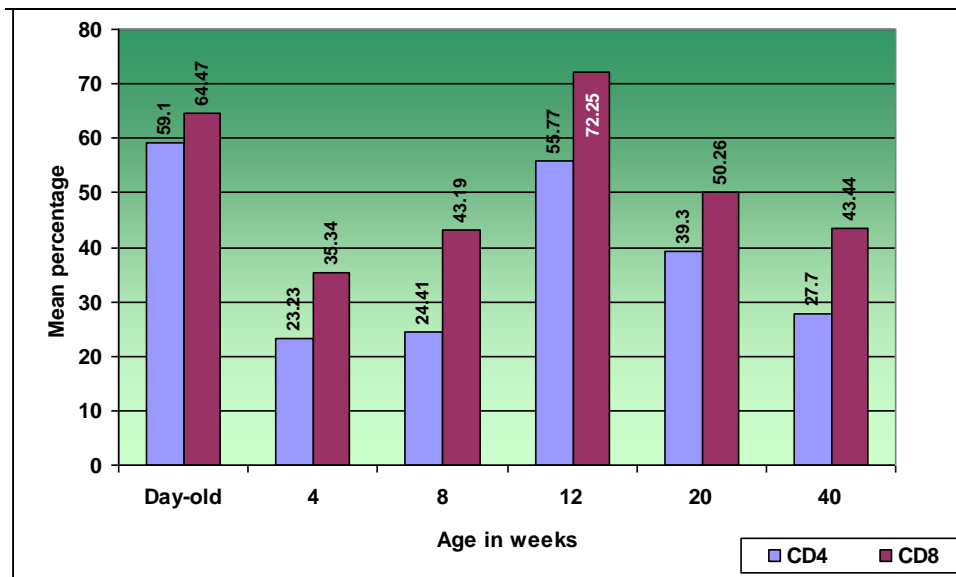
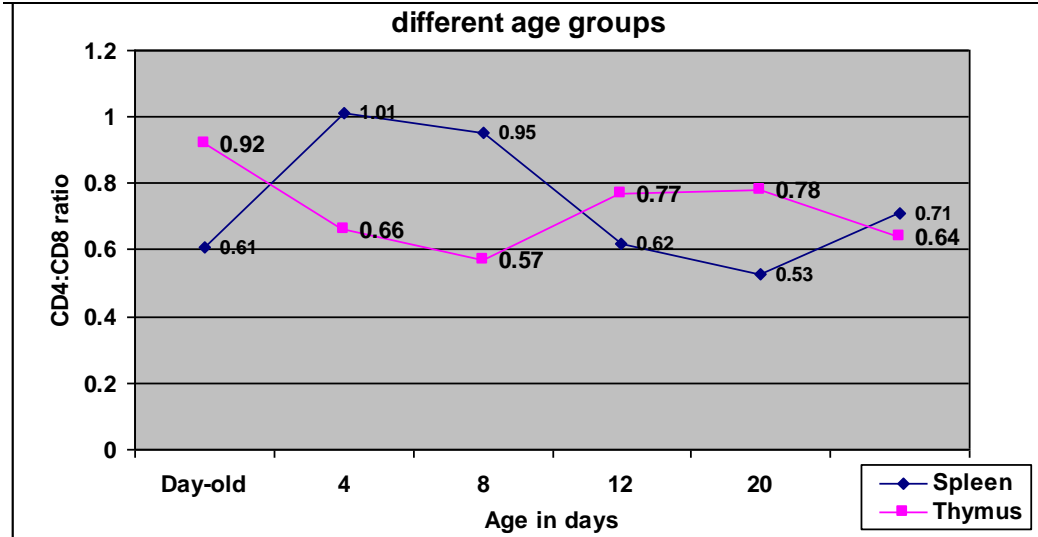


Fig.3 CD4 and CD8 T cell ratios in Spleen and Thymus of different age groups



Nevertheless, an increase in both the T cell population was detected at forty weeks of age. Bridle *et al.*, (2006) have detected an increase in the circulating CD4 and CD8 T cell populations by eighty weeks. Although we have not assessed the circulating T cell populations in our study, the increase in the population of these cells in spleen at two hundred days concurs with the findings of Bridle *et al.*, (2006). Among the two cell types studied, CD8 cells were always higher than the CD4 cells, with significant difference ( $P < 0.05$ ) at day one.

The distribution pattern of CD4 and CD8 cells in thymus was slightly different from that of spleen. Thymus had more CD4 cells on day one followed with twelve weeks of age. In contrast, CD8 cells were more on twelve weeks followed with day one. CD4 and CD8 cell populations were lowest on four weeks. Although we did not observe any set pattern of distribution of these cell types in the spleen and thymus of normal chicken, the results we have recorded concurs with some of the earlier reports (Davidson and Boyd, 1992) in that substantial populations of these cells could be detected at two and seven weeks of age.

In the present study the CD4 and CD8 ratio at four weeks of age was 1.01 in spleen and 0.67 in thymus whereas the reverse condition was recorded by Erf (1997) in spleen and thymus of commercial broilers and specific pathogen single comb white leghorn birds.

Similarly, CD4 and CD8 ratio in spleen was decreased from four weeks of age (1.01) to eight weeks of age (0.95). Erf *et al.*, (1998) recorded decreased in CD4 and CD8 ratio between two and seven weeks of age in commercial broiler chicken. Further Erf *et al.* (1998) also found that there was an increase CD4 and CD8 ration in thymus between two weeks (1.20) and seven weeks (2.30). A similar increase CD4 and CD8 ratio in thymus was observed in the present study between four weeks (0.67) and eight weeks (0.72).

A distinct difference was observed in the ratios of CD4 and CD8 cells in the spleen and thymus at different age. At day one the ratio was less in spleen and high in the thymus. However, at eight weeks, the CD4 and CD8 cell ratio was high in spleen and low in thymus. Nevertheless, the ratios narrowed down at forty weeks of age. The relationship between the mean CD4 and CD8 values in

spleen and thymus of different age groups were shown (Figure- 3).

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