

Original Research Article

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Status of Total Lymphocytes and T-Lymphocytes Population in Healthy Broiler Chicks

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ABSTRACT

The present study was carried out on 3-4 weeks old pathogen free broiler chicks (6 in numbers), maintained on standard feed from Ranchi Veterinary College (BAU) poultry farm . During the total lymphocyte count, lymphocytes appeared very distinctly from monocytes obtained from white buffy coat of mononuclear cells after centrifugation with Ficoll-paque by trypan blue dye exclusion technique with the help of haemocytometer. T-lymphocytes/T-cells showed characteristic greenish yellow fluorescence when stained with FITC (1:10 dilution) tagged antiserum raised against thymocytes. The mean of total lymphocytes & T-lymphocytes of 6 chicks taken as the blood values for time interval of one week on all days of examination were found to be highly significant ($p < 0.01$). T-lymphocytes were found to be about 70% of total lymphocytes in healthy chicks in all days of examination persistently; although their number (total lymphocytes and total T-lymphocytes) increases with age.

Keywords

Total lymphocyte,
T-lymphocyte,
Immune system,
Broiler chicks.

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Introduction

Broiler chicken being a good nutritive source of poultry meat at relatively low price has ranked higher position in the animal origin foodstuffs. The continuous increase in the economic importance of broiler chicks requires a better understanding of the development and function of immune system

in these birds. The avian immune system refers to the system of biological structures and cellular processes that protects birds from disease.

Lymphocytes are the main cellular components of immunity (Davison *et al.*, 1996); characterized by specificity, memory and heterogeneity. Specificity and memory

against an antigen are the common phenomena for the B-lymphocytes and T-lymphocytes but heterogeneity is only exhibited by T-lymphocytes.

Immunocompetence of an organism decides the susceptibility or resistance to an infectious disease in that organism. The immunocompetence was measured by counting of subsets of lymphocytes (T-lymphocytes & B-lymphocytes) population in chicken (Zekarias *et al.*, 2002). B-lymphocytes represents humoral immunity by producing antibodies, whereas T-lymphocytes represents cell mediated immunity by producing heterogenous population of cells (such as helper T-cells, cytotoxic T-cells, etc.).

Although the immune system of the chicken has been studied most broadly; avian immunology is complex and by no means it can be said complete, thus much research is still needed. The present study was performed to understand the status of total lymphocytes and T-lymphocytes population in healthy broiler chicks at different ages.

Materials and Methods

The present study was carried out on 3-4 weeks old pathogen free broiler chicks (6 in numbers), maintained on standard feed at experimental animal house of Ranchi Veterinary College, Ranchi.

Total lymphocyte count

Total lymphocyte count was carried out as per the method described by Prasad *et al.*, (2010) with slight modification was followed. 2 ml of heparinised & diluted blood was layered slowly over 1 ml of Ficoll-paque in centrifuge tubes, and centrifuged in a cooling centrifuge. The lymphocytes and monocytes formed white buffy layer, which was removed carefully and the volume of suspending fluid

was measured. The number of cells per unit volume of the suspension was counted by trypan blue dye exclusion technique as per the method described by Hudson and Hay (1978) and expressed as lymphocyte count per microlitre (μ l) of blood.

T-lymphocyte count: For the purpose of counting of T-lymphocyte; antiserum was produced according to Shivanandan and Maheshwaran (1980) method. The obtained sera were cross identified by double immunodiffusion test proposed by Ouchterlony *et al.*, (1986) after visualizing specific precipitation pattern.

The sera were adsorbed as per Albini and Wick (1974) techniques and isolated by Nowotny (1979) dialysis technique. Ultimately adsorbed and purified sera (immunoglobulins/antiserum against thymocytes) were conjugated to FITC (Fluorecein isothiocyanate) after their protein content estimation by Lowry *et al.*, (1951) method. According to Johnstone and Thorpe (1982) and Chauhan and Verma (1983); FITC was added to sera in 1:20 proportion on magnetic stirrer and left overnight at 4°C for complete conjugation; then conjugated sera were eluted through Sephadex G-25 column.

Direct fluorescent antibody technique for the lymphocytes and T-lymphocytes of broiler chicks on 4th, 5th, 6th and 7th weeks of age were studied for sheer count as referred by Chauhan and Verma (1983). Smears of lymphocytes were prepared with (1:10 dilution) FITC conjugated sera. Firstly, total number of lymphocytes was counted in a field under tungsten light; thereafter the number of fluorescing cells was determined in that very field under UV-light. Percentage of T-lymphocytes was calculated and the number of T-lymphocytes/ μ l blood was calculated accordingly to percentage of total lymphocytes count.

Results and Discussion

Lymphocytes appeared very distinctly from monocytes having centrally placed nucleus smaller than that of monocytes; obtained from white buffy coat of mononuclear cells after centrifugation with Ficoll-paque by trypan blue dye exclusion technique with the help of haemocytometer (Fig. 1).

T-lymphocytes/T-cells showed characteristic greenish yellow fluorescence when stained

with (1:10 dilution) FITC tagged antiserum raised against thymocytes in the UV light (Fig.2). The mean of total lymphocytes & T-lymphocytes of 6 chicks (Table 1.) taken as the blood values for time interval of one week on all days of examination were found to be highly significant ($p < 0.01$) as Student's t-test value. T-lymphocytes were about 70% of total lymphocytes in healthy chicks in all days of examination persistently; although their number increases with age is depicted by a bar column chart (Fig. 3).

Table.1 Total lymphocyte and total T-lymphocyte count* in broiler chicks

Results expressed as Mean±S.D				
Cell counting	Age of broiler chicks			
	4 th week	5 th week	6 th week	7 th week
Total lymphocytes	1717.5±283.26(6)	1892.5±295.29(6)	1909.2±295.88(6)	2117.5±309.09(6)
Total T-lymphocytes	1116.3±183.96(6)	1325.2±214.72(6)	1343.2±217.66(6)	1588.2±254.31(6)
t-test value	3.83**	5.13**	4.04**	3.52**

*Per µl of blood;(** $p < 0.01$);figures in brackets indicate the number of observations

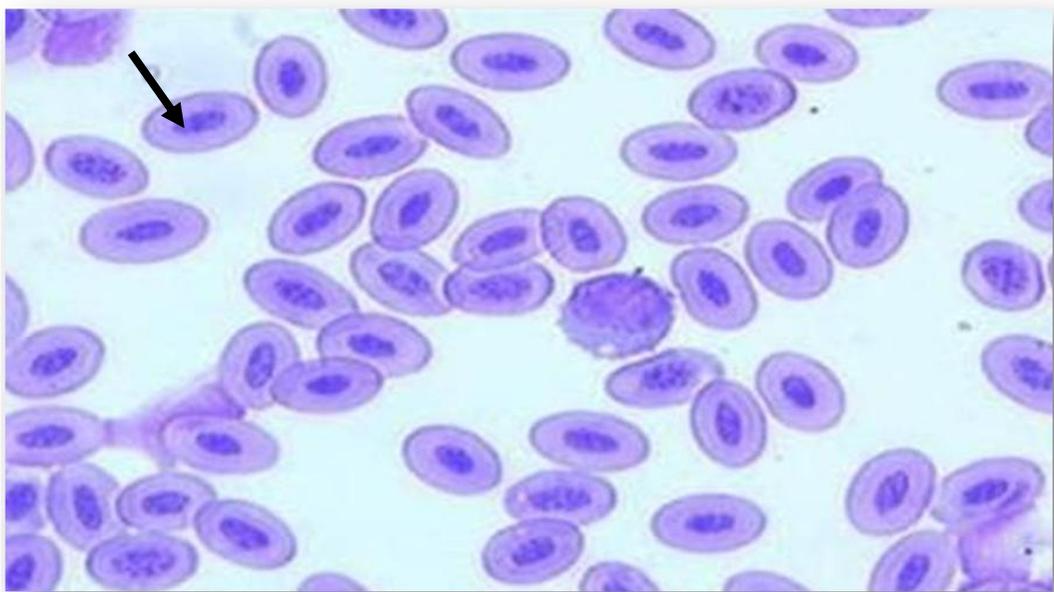


Fig.1 Arrow indicates dead lymphocyte (blue stained)

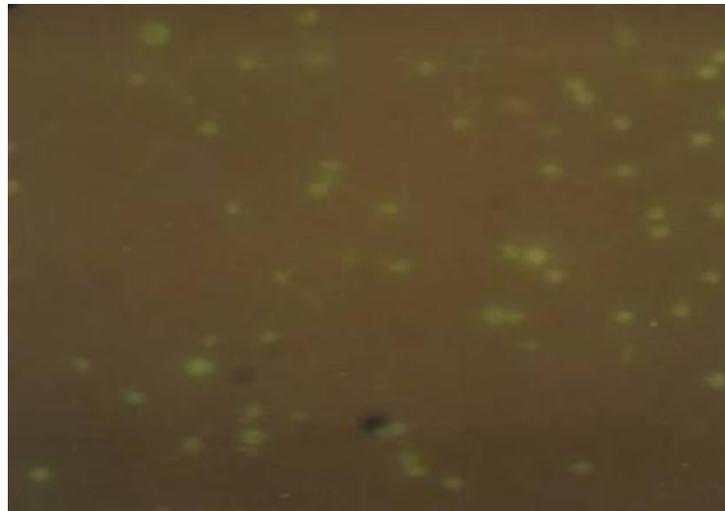


Fig.2 T-lymphocyte count in fluorescense microscope tagged with Anti thymocyte globulin conjugated with FITC (1:10 dilution)

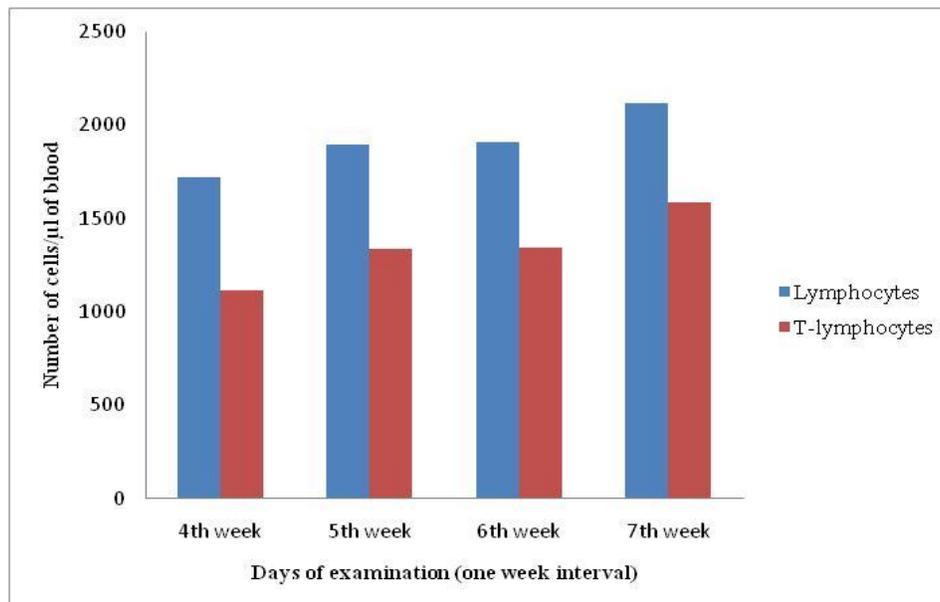


Fig.3 Bar column chart showing total lymphocytes and total T-lymphocytes population count

Lymphocytes appeared very distinctly from monocytes obtained from white buffy coat of mononuclear cells after centrifugation with Ficoll-paque by trypan blue dye exclusion technique with the help of haemocytometer (Fig. 1). On contrary to the finding; Pires *et al.*, (2007) stated that fasting of chicks as the important factor in avian leukocyte counts. The increase in feed restriction condition simultaneously decreases the leukocyte number.

Thus the variation of lymphocyte counts in the peripheral blood, if occurs; may be due to fasting of chicks. T-lymphocytes/T-cells showed characteristic greenish yellow fluorescence when stained with (1:10 dilution) FITC tagged antiserum raised against thymocytes (Fig.2). Walstra *et al.*, (1985) reported largest variation of T-lymphocytes in the peripheral blood of chicks as methodological errors by the use of Ficoll-paque. But our findings were different and we

stress on careful quantitative analysis of chicks peripheral blood for the yield of T-lymphocytes. The mean of total lymphocytes & T-lymphocytes of 6 chicks (Table 1.) taken as the blood values for time interval of one week on all days of examination were found to be highly significant ($p < 0.01$) as Student's t-test value.

T-lymphocytes were about 70% of total lymphocytes in healthy chicks in all days of examination persistently; although their number increases with age is depicted by a bar column chart (Fig. 3). Göbel *et al.*, (1994), reported that the peripheral blood lymphocyte populations are known to be under genetic control thus they prove to be an important biomarker for evaluating immunocompetence. It is the matter to argue that lymphocyte populations may have genetically diverged over the time, but unimmunized commercial broiler chickens are unlikely to be available.

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