Study of Blood Cells and Serum Proteins of Three Different Varieties of Fowls

Suprava Roy, Y. Vasudeva Rao

ABSTRACT
Background: In the present study attempts have been made to study the blood cells and serum proteins comparatively among three different varieties of fowls, namely Turkey, RIR and Kadaknath. Total RBC and WBC count and serum protein analysis were performed.

Methods: The total RBC and WBC were counted with the help of Neubauer’s Haemocytometer. The serum proteins were separated and analysed by native polyacrylamide gel electrophoresis (PAGE).

Result: The results showed that, among all the fowl varieties tested, the RBC counts were ranged from 3.01 to 4.46 millions/mm³ and the WBC counts were ranged from 18.26 to 21.78 thousands/mm³. The serum protein analysis revealed that some proteins are present in specific species and specific sex. Hence, these proteins may be counted as markers to determine the species and sex of the species.

Key words: Blood cell count, Kadaknath, RIR, Serum protein analysis, Turkey.

INTRODUCTION
Several varieties of fowls are reared in the poultry industry. Each variety of fowl have distinct physical characters which can be observed easily. Some of these distinct features are - life span, mature body weight, body shape, egg laying capacity, egg colour, egg size etc. Apart from these, chemical variations include proteins, vitamins, minerals, cholesterols content in meat and eggs. Kadaknath, also known as ‘Kala Masi’ has 10-12 years of lifespan. They are sexually matured at the age of 24 weeks. They lay 120-130 eggs per year and the colour of egg is light brown. The average weight of egg is 50 g. The bodyweight of male birds is about 2 kg, whereas the female is about 1.5 kg. Its meat is famous for its ‘black’ colour due to high amount of melanin content (Rao and Thomas 1984). The lifespan of RIR is about 10-14 years. They are sexually matured at the age of 24 weeks. They can lay 250-300 eggs per year and the colour of eggs is brown. The average weight of egg is 50 g. They have rectangular, relatively long bodies, typically dark red in colour. On an average, a male RIR weighs about 3.9kg and a female weigh about 2.9kg (Ekarius, 2007). The lifespan of turkey is about 10-12 years. They are sexually matured at the age of 30 weeks. They can lay 80-100 eggs per year and the colour of eggs is Creamy white with brown spots. The average weight of egg is 85 g (Marble and Margolf, 1936). The average weight of adult males is 7.6 kg and the average weight of adult females is 4.26 kg (Dunning and John, 1992).

Due to the dense rearing of the fowls, the chances of infections and mortalities are very high in the poultry. If a single bird is infected, it spreads easily and rapidly to the entire group of birds reared in a farm. As in all the animals, the fowl also has the immune system that can defend the normal threat posed by certain microbes. Though several varieties of fowls are reared in the poultry industry, which have distinct physical characters, we would like to study the blood cells counts and serum protein analysis for possible biochemical differences.

MATERIALS AND METHODS
Bird Selection
Three types of birds, i.e. Kadaknath, RIR and turkey are selected for this study. 12 birds of each type are taken for the study. Each group contain 3:1 female: male ratio, i.e., each group contain 9 females and 3 males. The age of both Kadaknath and Rhode Island Red (RIR) is 24 weeks and Turkey is about 30 weeks. The average body weight of Kadaknath female and male was 1.5 and 1.7 kg respectively. The average body weight of RIR female and male was 1.7 and 2.1 kg respectively. The average body weight of Turkey female and male was 3.9 and 4.2 kg respectively.

Housing and Rearing
The experimental house in which the chicks were kept was semi closed Reinforced Cement Structure with east – west direction. The housing dimensions were 25 m. length, 8.8
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m. width and 3.05 m height. The walls of the house on the northern and southern sides were built from bricks and cement. The floor and roof were made of concrete. No mechanical ventilation system was used but only natural ventilation system was allowed. The experiments were conducted during middle March to end June. The birds were reared at ‘Dairy and Poultry Farm’, Department of Animal Science (Poultry), Palli Siksha Bhavana, Visva-Bharati, Sriniketan. The climate is tropical, the average annual temperature and rainfall are 26.3°C and 1287 mm, respectively. The birds are fed with regular ration, which brought from epic (Kolkata). RIR and Kadaknath are fed with layer feed. Turkeys are fed with turkey feed. The feed method is as per standard.

All the experiments were performed, during March-April 2019, at the Department of Animal Science and the Biochemistry laboratory of Department of Soil Science and Ag. Chemistry, Institute of Agriculture, Visva-Bharati, Sriniketan, West Bengal, India.

Total RBC and WBC Count

Total Red Blood Cells (RBC) and White Blood Cells (WBC) were counted under the microscope using the standard procedure.

Blood collection and Serum preparation

Blood is collected from wing of each bird (i.e. Kadaknath, RIR and turkey) at morning in between 9 am to 10 am. Blood was collected in the clean sterilized 1.5 ml micro vial without any anti-coagulant and allowed to clot at room temperature for 30 min. serum was separated and collected after centrifuging the samples at 10000 g at 4°C for 15 min.

Separation of Serum Proteins by Native Polyacrylamide Gel Electrophoresis (PAGE)

Native Polyacrylamide Gel Electrophoresis (PAGE) of the serum sample was performed according to the method of Nondenaturing Polyacrylamide Gel Electrophoresis of Proteins (Walker, 2002). The molecular weight of each serum protein band was determined.

RESULTS AND DISCUSSION

Total RBC and WBC count

The mean ± SD of RBCs per mm³ in the blood of 3 different species, i.e., RIR, Kadaknath and Turkey are shown in Fig 1. Total RBC counts - in Turkey male and female fowls were 3.89 ± 0.15 and 3.01 ± 0.35 millions/mm³, respectively; in Kadaknath male and female fowls were 4.46 ± 0.11 and 3.43 ± 0.13 millions/mm³, respectively; in RIR male and female fowls were 4.02 ± 0.15 and 3.53 ± 0.10 millions/mm³, respectively. From the results it was observed that the RBC counts were always higher in males than their respective females. Among the different species, Kadaknath (male) has highest RBC counts among all birds studied. Though Kadaknath (male) found to possess highest RBC counts among all the males of different fowl species, however, Kadaknath (female) did not possess the highest RBC counts among all the females of different species. In the females, RIR was found to have higher RBC counts among all the females of different species. Nevertheless, except in turkey, all the females were found to possess nearly similar levels of RBC counts, i.e., 3.43, 3.53 million in Kadaknath (female) and RIR (female), respectively. And the Turkey (female) was having the least RBCs among all, i.e., 3.01 million only.

In this study, the observed total RBC values in Kadaknath male and female were 4.46 ± 0.11 and 3.43 ± 0.13 millions/mm³, respectively, is greater than the observation of Priya and Gomathy, (2008), which is 3.72 ± 0.03 and 3.26 ± 0.03 millions/mm³, respectively. The observed total RBC values in RIR male and female were 4.02 ± 0.15 and 3.53 ± 0.10 millions/mm³, respectively, is similar to the observation of Pandian et. al. (2012), which is 2.52 ±0.08 millions/mm³. Pandian et. al. (2012), have studied the RBC counts in Kadaknath, RIR and Turkey and found that the total RBC were higher in Kadaknath than RIR and Turkey, which is in agreement with our present study. In the present study also Kadaknath variety was found to have more RBC than Turkey and RIR varieties.

The mean ± SD of WBCs per mm³ in the blood of 3 different species, i.e., RIR, Kadaknath and Turkey are shown in Fig.2. From the results, it was observed that there was no significant difference in WBC counts between males and its respective females. The total WBC counts were shown to be similar in males and respective females. Among the different species, Kadaknath has highest WBC counts among all birds studied. The total WBC counts were ranged from 18,000 to 21000 among all the species tested. Except the Turkey species, the total WBC count was nearly similar, i.e., around 20,000. In Kadaknath (male and female) and RIR (male and female), the total WBC counts were around 20,000. In Turkey male and female, the total WBC counts were around 18,000. The Turkey was having the least WBCs.

Fig 1: Total RBC count in the male and female fowls of different varieties. The values represented are Mean±SD of 3 replicates.
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among all the species tested, similarly, it has the least number of WBCs among all the species tested, i.e., 18,000 only. Though the average body weight and the size of the Turkey is much higher than the rest of the fowl species, the WBC counts in the turkey were found to be significantly lower than the remaining species.

In this study, the observed total WBC values in RIR male and female were 20.48 ± 0.12 and 20.58 ± 0.75 thousand/mm³, respectively. The observed total WBC values in Kadaknath male and female were 21.78 ± 0.95 and 20.07 ± 0.30 thousand/mm³, respectively, is almost same as the observation of Preeti, et al., (2018), which is 26.90 ± 2.29 and 26.30 ± 1.76 thousand/mm³, respectively. The observed total WBC values in Turkey male and female were 18.26 ± 1.29 and 18.35 ± 1.14 thousand/mm³, respectively, which is higher than the observed values of Priya and Gomathy, (2008), i.e., 12.57 ± 0.05 and 11.99 ± 0.05 thousand/mm³, respectively.

It was observed that the Kadaknath breed excels in total RBC and WBC, which is indicative of better resistance of the breed to diseases. Hence, it may be considered as better vitality that could show resistance to disease conditions.

Separation of Proteins by Native Polyacrylamide Gel Electrophoresis (PAGE)

The serum from 3 verities of birds i.e., Kadaknath, RIR and Turkey were subjected to native polyacrylamide gel electrophoresis (Fig 3). The molecular weights of each separated protein band were determined.

Serum Protein analysis of Turkey male and female

From the native PAGE electrophoresis, 15 different proteins were found in the serum of Turkey, out of which 13 proteins are present in female fowl, whereas only 11 proteins were observed in male fowl (Fig. 4). The serum protein profile of the male fowl is different from the female fowl. The molecular weights (in kDa) of the 13 proteins present in the female Turkey were 305.7, 243.58, 221.6, 186.9, 151.79, 110.5, 78.3, 56.75, 42.73, 30.5, 23.77, 16.9 and 13.22 kDa, respectively. The molecular weights (in kDa) of the 11 proteins present in the male Turkey were 305.7, 243.58, 186.9, 138.09, 110.5, 91.07, 78.3, 42.73, 30.5, 23.77 and 16.90 kDa. It was observed that the Kadaknath breed excels in total RBC and WBC, which is indicative of better resistance of the breed to diseases. Hence, it may be considered as better vitality that could show resistance to disease conditions.

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16.9 kDa, respectively. Proteins 3, 5, 10 and 15 having molecular weights of 221.6, 151.79, 56.75 and 13.22 kDa were present only in female Kadaknath, but these proteins were absent in male Kadaknath. Proteins 6 and 8 having molecular weights of 138.09 and 91.07 kDa were present only in male Turkey, but these proteins were absent in female Turkey. Protein-1 and Protein-7 were present in high quantity in female Turkey than its male counterpart. Protein-12, Protein-13 and Protein-14 were present in high quantity in male Turkey than its female counterpart.

**Serum Protein analysis of Kadaknath male and female**

From the native PAGE electrophoresis, 16 different proteins were found in the serum of Kadaknath, out of which 13 proteins are present in female fowl, whereas only 12 proteins were observed in male fowl (Fig 5). The serum protein profile of the male fowl is different from the female fowl. The molecular weights (in kDa) of the 13 proteins present in the female Kadaknath were 305.7, 243.58, 221.6, 190.47, 154.69, 125.62, 120.96, 98.35, 45.22, 32.8, 23.77, 16.9 and 13.22 kDa, respectively. The molecular weights (in kDa) of the 12 proteins present in the male Kadaknath were 305.7, 243.58, 190.47, 148.15, 125.62, 100.11, 78.3, 45.22, 32.8, 23.77, 16.9 and 13.22, respectively. Proteins 3, 5, 8 and 10 having molecular weights of 221.6, 154.69, 120.96 and 98.35 kDa were present only in female Kadaknath, but these proteins are absent in male Kadaknath. Protein 6, 9 and 11 having molecular weights of 148.15, 100.11 and 78.3 kDa were present only in male Kadaknath, but these proteins are absent in female Kadaknath. Protein-1 was present in high quantity in female Kadaknath than its male counterpart. Protein-4 was present in high quantity in male Kadaknath than its female counterpart.

**Serum Protein analysis of RIR male and female**

From the native PAGE electrophoresis, 14 different proteins were found in the serum of RIR, out of which 12 proteins are present in female fowl, whereas only 11 proteins were observed in male fowl (Fig 6). The serum protein profile of the male fowl is different from the female fowl. The molecular weights (in kDa) of the 12 proteins present in the female RIR were 305.7, 243.58, 221.6, 190.47, 154.69, 116.47, 96.39, 45.22, 34.5, 23.77, 16.9 and 13.22 kDa, respectively. The molecular weights (in kDa) of the 11 proteins present in
Table 1: Different Serum Proteins Present in male and female fowls of Turkey, Kadaknath and RIR varieties.

| Protein   | Female Molecular weight (kD) | Male Molecular weight (kD) | Female Molecular weight (kD) | Male Molecular weight (kD) | Female Molecular weight (kD) | Male Molecular weight (kD) |
|-----------|------------------------------|---------------------------|------------------------------|---------------------------|------------------------------|---------------------------|
| Protein-1 | 305.70                       | 305.70                    | 305.70                       | 305.70                    | 305.70                       | 305.70                    |
| Protein-2 | 243.58                       | 243.58                    | 243.58                       | 243.58                    | 243.58                       | 243.58                    |
| Protein-3 | 221.60                       | -                         | 221.60                       | -                         | 221.60                       | -                         |
| Protein-4 | -                            | -                         | 190.47                       | 190.47                    | 190.47                       | 190.47                    |
| Protein-5 | 186.90                       | 186.90                    | -                            | -                         | -                            | -                         |
| Protein-6 | -                            | 154.69                    | -                            | 154.69                    | -                            | 154.69                    |
| Protein-7 | 151.79                       | -                         | -                            | -                         | -                            | -                         |
| Protein-8 | -                            | 148.15                    | -                            | 148.15                    | -                            | 148.15                    |
| Protein-9 | -                            | 138.09                    | -                            | -                         | -                            | 138.09                    |
| Protein-10| -                            | 125.62                    | -                            | 125.62                    | -                            | 125.62                    |
| Protein-11| -                            | 120.96                    | -                            | 120.96                    | -                            | 120.96                    |
| Protein-12| -                            | -                         | 116.47                       | 116.47                    | -                            | 116.47                    |
| Protein-13| 110.50                       | 110.50                    | -                            | -                         | -                            | -                         |
| Protein-14| -                            | -                         | -                            | 100.11                    | -                            | -                         |
| Protein-15| -                            | -                         | 98.35                        | -                         | -                            | 98.35                     |
| Protein-16| -                            | -                         | -                            | 96.39                     | -                            | 96.39                     |
| Protein-17| -                            | 91.07                     | -                            | -                         | -                            | -                         |
| Protein-18| 78.30                        | 78.30                     | -                            | 78.30                     | -                            | 78.30                     |
| Protein-19| 56.75                        | -                         | -                            | -                         | -                            | -                         |
| Protein-20| -                            | -                         | 45.22                        | 45.22                     | 45.22                        | 45.22                     |
| Protein-21| 42.73                        | 42.73                     | -                            | -                         | -                            | -                         |
| Protein-22| -                            | -                         | -                            | 34.05                     | -                            | 34.05                     |
| Protein-23| -                            | -                         | 32.80                        | 32.80                     | -                            | 32.80                     |
| Protein-24| 30.50                        | 30.50                     | -                            | -                         | -                            | -                         |
| Protein-25| 23.77                        | 23.77                     | 23.77                        | 23.77                     | 23.77                        | 23.77                     |
| Protein-26| 16.90                        | 16.90                     | 16.90                        | 16.90                     | 16.90                        | 16.90                     |
| Protein-27| 13.22                        | -                         | 13.22                        | 13.22                     | -                            | 13.22                     |

The RBC counts were always higher in males than the respective females of the same species. Among the different species, Kadaknath (male) has highest RBC counts among all birds studied. RIR was found to have higher RBC counts among the females of three different species tested. There was no significant difference in WBC counts in male and its respective female of the same species. Among the different species, Kadaknath has highest WBC counts among three varieties of fowls. Among all the species tested, Turkey was having the least number of RBCs and WBCs. The present study also illustrated the differences in the serum proteins among male and its female of the same species as well as it also illustrated the differences among different fowl species, i.e., Kadaknath, Turkey and RIR. There are some proteins which are present in specific species and specific sex. Hence, these proteins may be counted as markers to determine the species and sex of the species.

**CONCLUSION**

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