The Pharma Innovation Journal 2022; 11(2): 1825-1829

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(2): 1825-1829

www.thepharmajournal.com
Received: 07-12-2021
Accepted: 18-01-2022

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Effect of neem (*Azadirachta indica*) supplementation on hematological parameter of Kuroiler chicks

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Abstract
The present investigation was conducted to detect the effect of Neem (*Azardirechta indica*) supplementation on haematological parameters of Kuroiler chicks. One hundred twenty unsexed kuroiler chicks (day old) were used on a completely randomized design (CRD) in 4 treatments with 3 replicates, each consisting of 10 chicks. The treatments involved the control group (T_1) fed with chick starter feed and other group were fed on basal diet mixed with neem powder @ 2g, 4g, 6g in T_2, T_3, T_4 respectively. All other management practices were followed as per recommendation throughout the investigational period of 8 weeks. Blood samples were collected at the end of investigational period. The results of haematological parameter of treated group T_4 were significantly higher and showing good results among all groups control T_1 and treated group T_2, T_3 groups. Haematological values indicate that dry Neem leaves powder as an additive to feed has great potential for improving immune system response. Haemoglobin, PCV, TEC and TLC values in Kuroiler chicken and showed significant ($P< 0.05$) differences over the control. The results of haematological parameters of Kuroiler chicken at 56 days are presented in table 4.8. The results of haematological parameters showed significant ($P< 0.05$) differences in the values of Hb, PCV, TEC and TLC which were significantly higher in T_4 than control. The range of Haemoglobin and PCV were 10.77 (T_1) to 11.98 (T_4) g/dl and 29.33 (T_1) to 39.33 (T_4) %, respectively. Total erythrocytes count (TEC) and total leucocytes count (TLC) among treatment groups ranging from 1.98 (T_1) to 2.45 (T_3) millions/mm3 and 24.74 (T_1) to 27.71 (T_4) thousands/mm3, respectively. Lymphocyte, monocyte neutrophils, eosinophils and basophils count among control group and treatment groups ranging from 17.67(T_1) to 29.67 (T_4), 0.98 (T_1) to 2.00 (T_4), 65.33 (T_1) to 77.33 (T_4), 1.33 (T_1) to 4.33 (T_4) and 0.83 (T_1) to 0.99 (T_4).

Keywords: Haematological parameter, kuroiler chicks and neem

Introduction
India ranks 3rd in the world production of eggs and 5th in the production of broiler. (DHAD 2017-18). The poultry sector is one of the rare examples of socio-economic development, which attained its present advanced stage without much international aid and investment from the Five-Year-Plans. The total Poultry in the country is 851.81 million in 2019, increased by 16.8% over previous Census. He total Backyard Poultry in the country is 317.07 million in 2019, increased by 45.8% over previous Census. The total Commercial Poultry in the country is 534.74 million in 2019, increased by 4.5% over previous Census. Highest poultry population state in India is Tamil Nadu followed by Andhra Pradesh, Telangana, West Bengal, and Maharashtra.

The Kuroiler chicken is a dual-purpose hybrid breed developed in India. It was created by Vinod Kapur of Kegg Farms Private Ltd. in the early 1990s. And the name 'Kuroiler' is a portmanteau of Keggg and Broiler. A dual-purpose breed producing meat and eggs. Multi-coloured in appearance and highly preferred by smallholder farmers, also useful for camouflage. Hens attain 2.5 kg within 12 months, begin laying eggs at five to six months, and then lay 150–200 eggs during their 12–16month egg laying period, initially more than 20 eggs per month (Keggfarms).

Chicken meat and egg are gaining acceptance because of having high nutritional value, universal taste appeal, no religious taboo and being fairly economical. Egg is one such food commodity, which cannot be adulterated and contains protein of very high biological value. Poultry meat is an ideal food for children, young, adult, old people and those attempting to control their weight. It is easy to digest and contains high quality protein having abundance of all the essential amino acids required for optimum human nutrition.

Azadirachta indica, generally known as neem, nim tree or Indian lilac, is a tree in the sepia family Meliaceae. It is one of two species in the genus *Azadirachta*, and is native to the Indian subcontinent, *i.e.*, India, Nepal, Pakistan, Bangladesh, Sri Lanka, and Maldives. It is typically full-fledged in tropical and semi-tropical regions. Neem trees also grow in islands located in the southern part of Iran. Its fruits and seeds are the source of neem oil. Neem (*Azadirachta indica*) is an indigenous plant of Asian subcontinent known for its useful medicinal properties like antibacterial, antiviral, antifungal, antiprotozoal, hepatoprotective, immunomodulator and various other properties without showing any adverse effects (Kale *et al*., 2003 [9]; Sadekar *et al*., 1998) [20]. Neem preparations fed to laying hens have been reported by Sadre *et al*., (1984) and Gowda *et al*., (1998) to significantly reduce the content of haemoglobin, erythrocyte count and packed cell volume.

Materials and Methods

Experiment Site

The experiment was conducted at Poultry farm, S.K.N. College of Agriculture, Jobner District Jaipur, (Rajasthan, India). Geographically Jobner is located 45.0 km west of Jaipur at 26005' North latitude, 75028' East longitude and at an altitude of 427 meter above the mean sea level. The area falls in agro-climatic zone III–A (Semi-arid eastern plain zone of Rajasthan). The climate of this region is a typically semi-arid, characterized by extremes of temperature during both summers and winters.

Experimental Deign

The present investigation was conducted to study the effect of need feeding on haematological parameters of Kroiler chicken from (day old) to 8 weeks of age. For the present study 120, day old chicks of Kuroiler chicken were procured from department of Livestock Production and management. The chicks were randomly distributed into four treatment groups each having 30 chicks and each group were further divided into three replicates of 10 chicks each. Neem leaves for the experiment were procured from SKNCOA, campus. Neem leaves were dried in sunshine and crushed to make fine powder were mixed mixed properly at appropriate concentrations in the feed as specified for different treatments. T1 group was provided standard chick ration as per BIS (2007) specifications without any supplementation and served as control, T2 received standard chick ration with dry neem leaves powder supplementation in feed @ 2g/kg feed. T3 standard chick ration with dry neem leaves powder supplementation in feed @ 4g/kg and T4 standard chick ration with dry neem leaves powder supplementation in feed @ 6g/kg. The experimental birds were nearly equal in the live body weight at the start of the experiment. The experiment was extended up to 8 weeks of age. Feed and water were supplied adlibitum during the experimental periods. Chicks were grown in brooders with raised wire floors and exposed to 24 hours of constant light (12 hrs on day light and the rest on artificial lighting, using 40-watt bulbs). All chicks were kept under the same environmental and hygienic conditions. Live weight, body weight gain, feed consumption, feed conversion ratio, performance index, protein efficiency ratio and energy efficiency ratio were recorded during the experiment period.

Preparation of Neem leaf powder for feed

Neem leaf were dried in sunshine and crushed to make fine powder were mixed at appropriate concentration in feed as specified for different treatments.

Blood Parameters

Haematological analysis was carried out from the blood collected at the end of the experimental period. The analysis of blood samples was carried at laboratory of state government located at Disease Diagnostic Lab, Gopinath marg, Panch batti, Jaipur. The bird from each treatment was starved for twelve hours and blood sample was collected from the wing vein of the selected birds from each treatment group with the aid of needle and syringe. The blood was transferred immediately into a set of sterile plastic tubes with and without anti-coagulant for haematological test. The test tubes were held in slanting position for serum separation. The serum was centrifuged to remove the erythrocytes present, if any. The clear, non-haemolysed sera then collected in clean, dry and labelled vials.

The blood samples were analysed for Haemoglobin (Hb), Total erythrocyte count (TEC), Packed cell volume (PCV) and Total leucocyte count (TLC) as follows.

Determination of Haemoglobin Concentrations (Hb)

The N/10 hydrochloric acid (HCL) was taken in a graduated tube up to 2 marks with the help of a dropper. Well-homogenized blood sample was then drawn into the Sahli pipette up to 20 cm. mark. The tip of the pipette was wiped with sterile cotton and the blood of the pipette was immediately transferred into the graduated tube containing hydrochloric acid. This blood and acid were thoroughly mixed by stirring with a glass stirrer. There was a formation of acid hematin mixture in the tube by hemolysing red blood cells by the action of HCL. The tube containing acid hematin mixture was kept standing in the comparator for 5 minutes. After that distilled water was added drop by drop. The solution was mixed well with a glass stirrer until the colour of the mixture resembled to the standard colour of the comparator. The result was read in daylight by observing the height of the liquid in the tube considering the lower meniscus of the liquid column. The result was then expressed in g%. The above procedure was matched by the Hellige hemometer method as described by Lamberg and Rothstein (1977).

Determination of Total Erythrocyte Count (TEC)

The analysis of total erythrocyte count was done by Adopting method developed by Lamberg and Rothstein (1977). Well-mixed blood sample was drawn with red blood cell diluting pipette exactly up to 0.5 marks of the pipette. Outside of the tip of the pipette was wiped with cotton. Then the pipette was immediately filled with the red cell diluting fluid (Hayem's solution) up to 101 marks. The free end of the pipette was wrapped around with the rubber tube stretching to both the ends and held with thumb and middle finger. The content of the pipette was mixed thoroughly by shaking with 8-knot motion for 3-5 minutes. Then the counting chamber was placed with special cover glass under microscope using low power (10x) objectives. After discarding two drops of fluid from the pipette, a small drop was placed to the edge of the cover glass on the counting chamber as the entire area under the cover glass was filled by the fluid. One-minute time was

spared to allow the cells to settle on the chamber under the cover glass. Taking 5 larger squares (4 in the 4 corners and the central one) of the central large square, the cells were counted from all the 80 small squares (16 x 5) under high power objectives (40x). After completion of counting, the total number of RBC was calculated as number of cells counted x 10,000 and the result was expressed in million/µl of blood.

Determination of Total Leukocyte Count (TLC)

The principals involved in enumeration of Total Leukocyte Count were almost same to those of erythrocytes. Here the leukocyte diluting fluid was used N/10 HCl. Well mixed blood was drawn upto the 0.5 mark of white blood cell pipette. The diluting fluid was filled upto the 11 mark of the pipette and the contents were thoroughly mixed for 2 minutes. Two drops of contents were discarded and counting chamber was then filled in the same way as in the RBC count. The counting chamber was placed under the microscope and examined under low power objective (10x). The leukocytes in the large squares (each 1 square mm) of the counting chamber were counted. The number of W. B. C. was calculated as follows:

Number of WBC = No. of cell counted x 10 and the result is expressed in thousand per cu mm.

3.7.5.4 Determination of Packed Cell Volume (PCV)

The citrated well mixed blood sample was drawn into special loading pipette (Wintrobe pipette). The tip of the pipette was inserted up to the bottom of a clean, dry Wintrobe haematocrit tube. Then the Wintrobe tube was filled from the bottom by pressing the rubber bulb of the pipette. As blood came out, the pipette was slowly withdrawn but pressure was continued on the rubber bulb of the pipette so as to exclude air bubbles. The tip of the pipette was tried to keep under the rising column of blood to avoid foaming and the tube was filled exactly to the 10 cm mark. Then the Wintrobe hematocrit tube was placed in the centrifuge machine and was centrifuged for 30 minutes at 3000 rpm. Then, the PCV was recorded by reading the graduation mark; the per cent volume occupied by the PCV was calculated by using the following formula as described by Lamberg and Rothstein (1977).

$$\text{PCV (\%)} = \frac{\text{Height of the red cell volume in cm}}{\text{High of total blood in cm}} \text{x } 100$$

Results and Discussion

Haematological Parameters

The outcomes of haematological parameters of Kuroiler chicken at 56 days are presented in table. The results of haematological parameters showed significant ($P< 0.05$) differences in the values of Hb, PCV, TEC and TLC which were significantly higher in T_4 than control.

The range of Haemoglobin and PCV were 10.77 (T_1) to 11.98 (T_4) g/dl and 29.33 (T_1) to 39.33 (T_4) %, respectively. Total erythrocytes count (TEC) and total leucocytes count (TLC) among treatment groups ranging from 1.98 (T_1) to 2.45 (T_3) millions/mm3 and 24.74 (T_1) to 27.71 (T_4) thousands/mm3, respectively. Lymphocyte, monocyte neutrophils, eosinophils and basophils count among control group and treatment groups ranging from 17.67(T_1) to 29.67 (T_4), 0.98 (T_1) to 2.00 (T_4), 65.33 (T_1) to 77.33 (T_4), 1.33 (T_1) to 4.33 (T_4) and 0.83 (T_1) to 0.99 (T_4).

The result of blood parameter in investigation were found similar, reported by Obikanonu *et al*. (2012), Beg *et al*. (2018), Khatun *et al*. (2013) [11], Pandian *et al*. (2012) [18] and Ubua *et al*. (2018) [26]. These Values are in agreement with findings by Bonsu *et al*. (2012) [7], Sarker *et al*. (2014) [21], Nayka *et al*. (2013), Ansari *et al*. (2012) [5], Wankar *et al*. (2008) [27], Akanmu *et al*. (2012) [2] and Anurag *et al*. (2018) [6].

Acknowledgment

I cannot express enough thanks to my almighty, my Dean Dr. A.K. Gupta, SKNCOA Jobner, my advisor Dr. Mahesh Datt professor and Head, Department of Livestock Production Management SKNCOA Jobner and my committee members Dr. R.P. Jat Dean & professor, Department of Livestock Production and Management in, college of agriculture Jhilai (Newai), Dr. M.K. Sharma, Assoc. Professor, Department of statistics, maths and computer science, Dr. J.P. Yadav, Department of Extension Education SKNCOA Jobner for continuously supporting me throughout my research work. The completion of research work could not have been possible without their support and assistance. Heartly thankful to my parents for supporting and encouraging me throughout the research work, I am really thankful and blessed.

Table 1: Effect of neem on mean haematological parameter of Kuroiler chicken at 56 day

Parameters	Units	T_1	T_2	T_3	T_4
Hb	g/dl	10.77±0.01	11.69±0.02	12.02±0.02	11.98±0.02
PVC	%	29.33±0.67	31.33±0.67	35.33±0.89	39.33±1.20
TEC	Millions / mm^3	1.98±0.003	2.12±0.015	2.30±0.029	2.45±0.012
TLC	Thousand/ mm^3	24.74±0.67	24.16±0.32	27.06±0.03	27.71±0.02
Lymphocyte	%	17.67±0.88	22.67±0.33	24.67±0.33	29.67±2.33
Monocyte	%	0.98±0.015	1.00±0.01	1.33±0.33	2.00±0.00
Neutrophils	%	65.33±2.18	71.00±0.57	71.33±0.88	77.33±1.20
Eosinophil	%	1.33±0.33	2.33±0.33	3.67±0.33	4.33±0.33
Basophils	%	0.83±0.018	0.89±0.018	0.97±0.018	0.99±0.007

Conclusion

It concluded that the outcomes of haematological parameter of treated group T4 were significantly higher and showing good results among all groups control T1 and treated group T2, T3 groups. Haematological values indicate that dry Neem leaves powder as an additive to feed has great potential for improving immune system response. Haemoglobin, PCV, TEC and TLC values in Kuroiler chicken and showed significant ($P< 0.05$) differences over the control.

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