



A Hematological study on the effect of environmental stress on cockerel bird

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Abstract: The effects of pollution on cockerel birds, which have their own distinct physiological and hematological features, have not received as much attention as they deserve. In my study, Hematology and development performance in birds like Cockerels are negatively impacted by this sort of pollution. Its sound waves also have an effect on the inner ear hair cells that translate between various pitch ranges. Cockerels, or male chickens, serve an important purpose in farming and poultry production. Not only can you consume them, but they also help increase the variety of chicken breeds available. Their well-being is, therefore, essential to the continued success of these sectors. Hematological studies were performed on the blood drawn from the brachial veins of both the control and experimental birds the next day, following the protocol described by Singh et al. (2003). In present investigation, I have studied hazards of generator caused in the form of air pollution and sound pollution. This type of pollution adversely affects the hematology of avian species as Cockerel birds and their growth performance. Beside, its sound also affects on the inner ear hair cells which are responsible for receiving sound of different frequencies.

Keywords: hematological study, environmental stress, cockerel bird, pollution, development performance, sound pollution, avian species, growth performance

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INTRODUCTION

The term "air" refers to the planetary envelope of various gases. We are protected from the potentially disastrous effects of space travel. It reaches out to a distance of 1,600 km from Earth and is rich in all the necessary ingredients for life. Nitrogen (N), oxygen (O₂), carbon dioxide (CO₂), inert gases, and trace amounts of contaminants make up the bulk of the mixture. The most vital gas for all living things is oxygen, and the most vital gas for sustaining producers, on which all heterotrophs rely, is carbon dioxide. At the ocean's surface, air exerts a pressure of 7 kilograms per square centimeter (kg/cm²). It is denser near sea surface but gets lighter with increasing height. In addition to being an insulator and playing a role in the biogeochemical cycle, it also serves as a cosmic ray absorber. (Bedanova, E 2007)

A significant portion of the air pollution linked to human cancer, heart and lung damage, and cognitive decline comes from diesel combustion emissions. Diesel exhaust also includes Nanoparticles, which have significant health consequences, and are as yet little understood. Diesel soot and aerosols such as ash particulates, metallic abrasion particles, sulfates, and silicates make up diesel particulate matter (DPM), also known as diesel exhaust particles (DEP). Most DPM is in the invisible sub micrometer range of 100 nanometers, also known as ultrafine particles (UFP) or PM_{0.1}, when discharged into the environment. (Khan, W.A; 2002)

Generators are a major source of both air and noise pollution. It creates a sound of the strength which diminishes with increase in the distance from the sources. It has been measured that a 5kva generator,

when placed 2 feet away, emits noise at a level of 60–70 dB. There is evidence that it also has an effect on sensory organs like the inner ear.

Diesel exhaust mostly contains very small particles. Small inhaled particles might potentially lodge deep into the lungs. Because of their rough surfaces, these particles readily bond with other chemicals in the environment, enhancing the dangers associated with inhalation.

Acute short-term symptoms associated with exposure include headache, dizziness, lightheadedness, nausea, coughing, difficult or laborious breathing, chest tightness, and eye, nose, and throat irritation. Heart disease, lung cancer, and other dangerous conditions may develop with prolonged exposure to a hazardous substance. Decreased brain function in older males was linked to exposure to ambient traffic-related air pollution. (Bedanova, E 2007)

In this study, I have studied the air pollution and noise pollution created by diesel generators which is quite harmful pollution. An increasing number of people are worried about human-caused air pollution. The quality of the air in dense metropolitan areas and near significant emission sources is negatively impacted by human activity, even though many of the gaseous contaminants are also generated by nature. Population growth, forest loss, fossil fuel burning (including fire), fast industrialization and urbanization, agricultural and industrial operations, conflicts, and vehicle and generator emissions all contribute significantly to man-made air pollution. Generators are a major source of both air and noise pollution. It creates a sound of the strength which diminishes with increase in the distance from the sources. It has been measured that a 5kva generator, when placed 2 feet away, emits noise at a level of 60–70 dB. There is evidence that it also has an effect on sensory organs like the inner ear. (Khan, T.A 2005)

Blood is the transport vehicle for the body. It's made up of plasma, a liquid intercellular material, and other formed components floating in solution. The erythrocytes, leucocytes, and thrombocytes, which are the mammalian equivalent of platelets, are the produced components. There are a wide array of molecules and components carried by blood. Large erythrocytes have an oval shape and a flat nucleus. The nucleus is often found in the center of the cell and is around 12 microns in length and 7 microns in breadth. Hemoglobin, an iron molecule, delivers oxygen and gives blood its characteristic red color. The erythrocytes' job is to shuttle oxygen from the lungs to the tissues and carbon dioxide back to the lungs. Red bone marrow is where the erythrocytes are made. There are around 2.5 million erythrocytes per milliliter of blood in an adult chicken (Campbell, T.W., 1995). Blood samples may be centrifuged at 3000 rpm for 15 to 20 minutes to determine cell volume. When the cells and plasma are separated, the cellular volume may be determined. (Oguz, K 1988)

The haemocrit is the volume of blood cells, which are mostly erythrocytes but also include some leucocytes and some trapped plasma. Because of the trapped plasma, there is a about 5% margin of error. Nucleated and amoeboid in shape, leucocytes have a clear cytoplasm. Others are non-granular, while yet others contain granules of varying sizes in their cytoplasm. Leucocytes are often categorized as granulocytes or agranulocytes based on whether or not they contain granules. The vast majority engage in phagocytosis. Therefore, the form varies, albeit it is often a sphere. Despite their apparent multi-lobedness, they only have a single nucleus. Leucocytes originate in the bone marrow, spleen, and lymphoid tissue. Approximately 30,000 per milliliter (ml) are found in mature birds, but only 10,000 per ml are found in

day-old hens (Campbell, T.W, 1995).

LITERATURE REVIEW

Llacuna et al. (1996) investigated the effects of SO₂ and NO_x in the environment on the haematological and plasma parameters of three different species of passerine birds and published their findings. For both the *Parus major* and the *Emberiza cia*, a decrease in RBC was seen, whereas an increase in MCV and MHC was documented for both species. In addition to this, *Emberiza* was found to have increased levels of pre-albumins and decreased levels of globulins. In *Turdus merula*, a reduction in body mass and an increase in transamine levels (GOT and GPT) have been shown to have a link with one another. Matooane et al. published a risk evaluation of sulfur dioxide pollution on human health in south Durban, South Africa, in 2003. The study was conducted in South Africa. Kleinman et al. (1989) conducted research on the effects of short-term exposure to carbon monoxide on individuals who suffered from coronary artery disease. Strom et al. (1984) investigated the effects that inhaling high amounts of diesel exhaust had on the cellular defenses found in the lungs of test subjects.

Oguz and Baskurt, 1988; Baskurt, 1988; Baskurt et al. 1990; Dikmenoglu et al. 1990) There has only been a limited amount of study done on the impact that NO_x, SO₂, and particulates from coal fueled power stations have on the haematological parameters of animals in their natural environment. On the other hand, when tiny animals were given large doses of SO₂ in the laboratory, they exhibited signs of haematological change.

Misaki et al. (2008), organic extracts of road dust and diesel exhaust particles both have the ability to function as aryl hydrocarbon receptor ligands and estrogen receptor ligands, respectively. It was hypothesized by Yamane and colleagues in 2007 that peptides and very small quantities of nitrogenous compounds govern the stress responses of newborn chicks. The effects of heat stress on the haematological parameters of broiler chicks were investigated by Khan et al. (2002). A dry particle aerosol generator with a minimal sample consumption was proposed for use with nasal inhalation exposure by Ledbetter (2002).

Morgan et al. (1980) performed hematological experiments and observed a reduction in the amount of hemoglobin, which may be connected to a decrease in the number of RBC and, as a consequence, suggests induction of an anemic state due to toxic effects.

Yalcin et al. (2003), the adverse effects of heat stress in broilers may be lessened by the use of early training and the restriction of feed intake during subsequent heat stress. When compared to broiler chickens, Guinea fowl have significantly higher levels of hemoglobin, basophils, monocytes, and neutrophils, as stated by Singh et al. (2003).

Arunachalam et al. (2003) looked at the hematological changes that occurred in broiler chickens after they were infected with eggs from the parasite *Ascaridia galli*.

According to the findings of a research that was carried out by Gangwar et al. (2006), the amount of noise that was present in the Kaithal Metropolitan City was just marginally higher than the limit that was established by the Central Pollution Control Board of India.

Amariki et al. (2009) investigated the effect that the flame and fumes from a refined petroleum product (kerosene) had on the output of broiler chickens.

Akporhwarho et al. (2011) cockerels that were reared in an intensive system may be more sensitive to the adverse effects of drinking water that was polluted with crude oil.

Mohanraj and Azeez (2004), the presence of fine particulate matter is a risk factor for premature death as well as mortality from cardiovascular disease and lung cancer. The beginning of the industrial period, urbanization, and the fast development of India's vehicle fleet have all contributed to a rapid decline in the country's air quality, which has been going on for quite some time. There has been a worrying rise in the amount of particle matter in cities all around the globe. In spite of the dishearteningly large statistics, there simply have not been sufficient research, notably in the field of environmental epidemiology. It is imperative that immediate action be taken in order to increase the accuracy with which morbidity is documented.

METHOD'S AND METHODOLOGY

Experimental Model

Cockerel birds were separated into two groups' viz. Experimental and control. There were six baby chicks in each group. Each experimental group was exposed to Diesel exhaust (DE) in a cage for 7, 4, 21, and 28 days, with exposure lasting 30 minutes on some days and 60 minutes on others. Each control group had the same number of birds that were released into the open air.

- **Experimental design**

1. Control group - Each set of six birds was given access to outside air as a control.
2. Six birds in each of the first, second, and third groups were subjected to Diesel Exhaust (DE) at distances of 2 feet, 6 feet, and 10 feet for 30 minutes for periods of 7, 14, 21, and 28 days.
3. Different groups of birds (IV, V°, and VI) were subjected to 60 minutes of exposure to diesel exhaust (DE) at distances of 2, 6, and 10 feet for 7, 14, 21, and 28 days.
4. Therefore, in the current study, we conducted a pair of experiments.
5. Before feeding the birds in each trial, blood was taken from the cockerel birds' brachial veins the next morning.

Haematological Studies

The following are the results of the conventional analysis of hematological parameters:

- **Total erythrocyte count (TEC)**

The total erythrocyte count ($1 \times 10^6/p1$) was determined using the technique developed by Natt and Herrick. New blood was taken into a normal blood dilution pipette to the 0.5 mark, and then methyl violet 2B diluent was added until the 10lmark.

Table 1: Natt and Herrick, 1952 (Methyl Violet 2B) Diluent

NaCl	3.88g
Na ₂ SO ₄	2.50
Na ₂ HPO ₄ • 12H ₂ O	2.91g
KH ₂ PO ₄	0.25g
Formalin (37%)	7.50ml
Methyl Violet 2B	0.10g

“This yields a final dilution 1:200; the pipette was then quickly shaken, wiped, and the Counting chamber of a Neubauer hemocytometer charged. The number of red blood cells was counted using a 40X objective after some time for the sample to settle.” The TEC per micro liter was calculated by counting the number of erythrocytes in the center and four corner squares and then multiplying that number by 106.

- **Hemoglobin Concentration (Hb %)**

20µl of well-mixed blood was added to 5ml of Drabkin's reagent to determine the hemoglobin concentration.

- **Packed cell volume (PCV %)**

Anticogluant microhematocrit tubes (standard, 75 mm in diameter) were used for blood collection. A standard Microhematocrit graphic reader was used to analyze the results of 12,000xg centrifugation for 5 minutes.

- **Total Leucocyte Count (TLC)**

The Natt and Herrick technique of TLC (1x10⁶/p1') was used (Natt, MP, and Herrick, CA 1952). Leucocytes were counted using a modified Neubauer hemocytometer with sixteen big counting wells:

TLC/ microlitre (Number of leucocytes in 16 squares) x 100

- **Differential Leucocyte Count**

A drop of blood was then put on a slide that had been previously cleaned. A coverslip was put on top of the blood on the microscope slide while it was held between the fingers, and then removed horizontally as the blood spread. The slides were then air-dried before being stained with Leishman. Leukocyte differential numbers (1x10³/p1') were determined using standard procedures.

- **Erythrocyte Sedimentation Count (ESR in mm/hr)**

Renewed Abstinence A wintrobe tube was filled to the zero mark with EDTA-anticoagulated, undiluted blood using a Pasteur pipette. The tube was positioned perfectly vertically. After one hour, the erythrocyte column height was measured in millimeters.

RESULTS AND DISCUSSION

Haematological Studies

Cockerel birds exposed to diesel exhaust (DE) for 7, 14, and 28 days at distances of 2 feet, 6 feet, and 10 feet for 30 minutes showed the following hematological alterations.

Hemoglobin concentration

A. Cockerel birds' Hemoglobin content (gm%) after being exposed to diesel exhaust (DE) for 30 minutes.

Table 1 provides information on the Hemoglobin concentration in the three test groups (I, II, and III) and the results of the statistical analysis. Group I and Group- II both had lower hemoglobin levels than the control group. The decrease in Group I after 7 and 21 days of exposure to generator-emitted diesel exhaust (DE) was statistically significant at the 1% level ($p < 0.01$).

After 7 and 14 days, the difference between Group-II and Group-I was statistically significant ($p < 0.05$). At the 1% level ($p < 0.01$), it was likewise significant in group-III. Duration: 28 days.

Table 1: Compared to a control group, the Hb cone. (gram/dl) of birds exposed to diesel exhaust (DE) from a 5kva generator at 2 feet, 6 feet, and 10 feet for 30 minutes.

Length of period Days	Control group mean \pm S.E. Control	Experimental group mean \pm S.E.		
		Group-IV (2ft)	Group-V (6ft)	Group-VI (10ft)
7	10.94 \pm 0.14	8.29 \pm 0.58a	7.73 \pm 0.08a	9.68 \pm 0.27
14	10.44 \pm 0.23	8.19 \pm 0.56a	8.44 \pm 0.64	9.89 \pm 0.35
21	10.93 \pm 0.05	8.65 \pm 0.85	7.36 \pm 0.63b	9.24 \pm 0.33
28	10.74 \pm 0.05	8.49 \pm 0.67b	9.52 \pm 0.01	8.71 \pm 0.45b

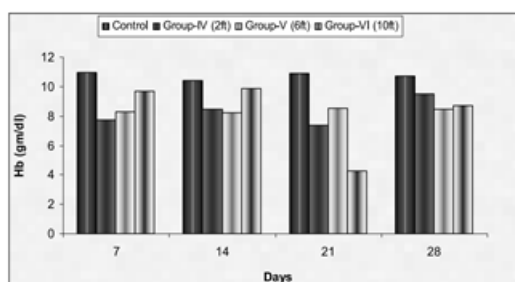


Figure 1: Diesel exhaust (DE) from a 5kva generator was tested on the hemoglobin (Hb) of exposed cockerel birds at 2 feet, 6 feet, and 10 feet for 30 minutes. contrasted with the placebo group.

B. Cockerel birds' Hemoglobin content (gm%) after being exposed to diesel exhaust (DE) for 1 hour.

“Table presents the statistical analysis and data on Hemoglobin concentration in the exposed groups (IV, V, and VI). Groups IV and V, which were exposed to diesel exhaust (DE), had lower hemoglobin concentrations than the control group.” The decrease was statistically significant at 5% level ($p < 0.05$) in Group IV for 7 days, 21 days and 28 days. “Both at the 2-week and 28-day marks, Group-VI showed statistical significance at the 1% level ($p < 0.01$). After 7 and 28 days, values in Group V and IV were likewise considerably at 1% ($p < 0.01$).

Table 2 : Exposed birds' hemoglobin (Hb) levels were measured before and after being exposed to diesel exhaust (DE) from a 5kva generator at distances of 2 feet, 6 feet, and 10 feet for one hour.

Length of period Days	Control group mean + S.E.	Experimental group mean + S.E.		
	Control	Group-IV (2ft)	Group-V (6ft)	Group-VI (10 ft)
7	10.41±0.20	7.91±0.80a	8.043±0.29a	9.32±0.09
14	10.60±0.30	9.43±0.01b	9.30±0.15b	9.37±0.12b
21	10.32±0.05	7.51±0.07a	8.43±0.83	8.96±0.36
28	10.87±0.07	7.96±0.68a	7.35±0.24b	7.08±0.66b

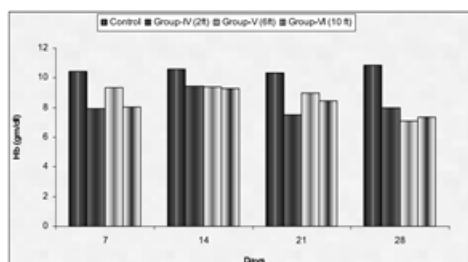


Figure 2: Birds' hemoglobin levels were measured before and after exposure to diesel exhaust (DE) from a 5kva generator at two, six, and ten feet per hour.

Packed cell volume

A. Cockerel bird PCV (packed cell volume) after 30 minutes of exposure to diesel exhaust

Table displays the packed cell volume (PCV%) data together with statistical analysis. Cockerel birds in Groups I, II, and III all had significantly lower PCV than the control group. On day 7, the difference between Group I and Group III was statistically significant at the 0.1% level ($p=0.001$), and on day 4, at the 1% level ($p=0.01$). Group II had a level of 0.1% ($p=0.001$). At the 0.1% level ($p=0.001$), on day 21 days, it was significant for all groups (I, III, and II). When comparing Groups I and II to the control group after 28 days of exposure, there was a statistically significant difference at the 1% level ($p=0.01$).

Table 3 : Cockerel subjected to diesel exhaust (DE) at 2 feet, 6 feet, and 10 feet for 30 minutes. Effect on packed cell volume (percent). When compared to the placebo group.

Length of period Days	Control group mean + S.E.	Experimental group mean + S.E.		
	Control	Group-I (2ft)	Group-II (6ft)	Group-III (10 ft)
7	32.10±0.01	29.29±0.40c	29.28±0.41c	28.86±0.23c
14	34.71±0.18	31.26±0.43b	30.91±0.66c	31.19±0.11b
21	36.49±0.13	29.60±0.85c	30.91±0.66c	31.20±0.11c
28	34.22±0.09	27.73±0.93b	30.80±1.91	27.74±0.93b

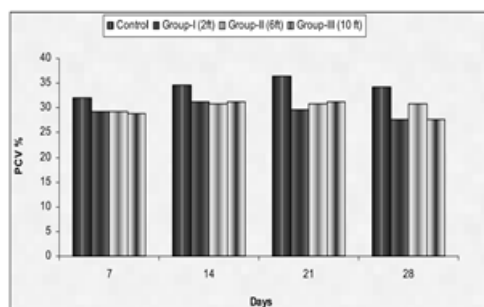


Figure 3: Diesel exhaust (DE) from a 5kva generator was tested on the packed cell volume (PCV%) of cockerel birds at exposure levels of 2 feet, 6 feet, and 10 feet for 30 minutes.

B. Cockerel birds' packed cell volume (PCV%) after being exposed to diesel exhaust (DE) for 1 hour.

Table displays the statistical evaluation of the packed cell volume (PCV%) data. Group IV shows a significant decline ($p < 0.01$) at the 1% level, whereas Groups V and VI show significant decreases ($p < 0.05$) at the 5% level. When compared to the control group, Groups IV, V, and VI had a 1% reduction over the course of 7 days ($p < 0.01$). On day 21, the difference between Group V and the control group was 5% ($p < 0.05$), and on day 28, the difference was 0.1% ($p < 0.001$).

Table 4: Diesel exhaust (DE) from a 5kva generator was tested on the packed cell volume (PCV%) of cockerel birds at exposure levels of 2 feet, 6 feet, and 10 feet for one hour.

Length of period Days	Control group mean + S.E.	Experimental group mean + S.E.		
		Group-I (2ft)	Group-II (6ft)	Group-III (10 ft)
7	31.41+0.35	28.32+0.71b	29.28+0.41a	28.86+0.23a
14	34.65+0.14	31.26+0.42b	30.91+0.66b	30.73+0.36b
21	35.90+0.01	32.26+0.64	31.38+1.42a	35.59+0.86
28	34.56+0.22	30.19+0.47c	29.00+0.28c	28.21+0.58c

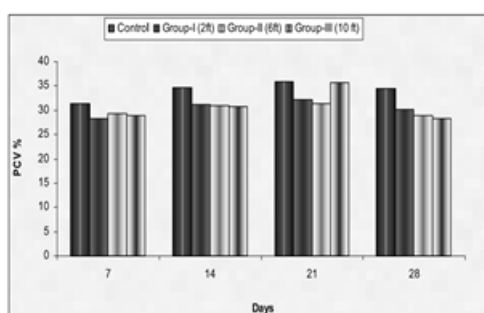


Figure 4: Diesel exhaust (DE) from a diesel generator was tested on the packed cell volume (PCV%) of cockerel birds at exposure levels of 2 feet, 6 feet, and 10 feet per hour.

OBSERVATION

Hemoglobin concentration (Hb %): The exposed groups had significantly lower hemoglobin concentrations than the control group ($p < 0.05$).

Packed cell volume (PCV %): In addition, the packed cell volume of cockerel birds was considerably

lower in the exposed groups compared to the control group (p 0.05).

Erythrocyte sedimentation rate (mm/hr): The total number of erythrocytes in the birds exposed to the substance was significantly lower than that of the control group (p 0.01).

Total erythrocyte count ($1 \times 10^6/\mu\text{l}$): The total number of erythrocytes in the birds exposed to the substance was significantly lower than that of the control group (p 0.01).

Total leukocyte count ($1 \times 10^3/\mu\text{l}$): Exposed groups had higher total leukocyte counts than control groups, by a significant margin (p0.05).

Differential leukocyte count ($1 \times 10^3/\mu\text{l}$): Exposure substantially increased the number of all types of leukocytes compared to the control group (p0.01).

CONCLUSION

This discovery is consistent with the conclusions drawn from hematological studies by Morgan et al. (1980), who discovered a drop in haemoglobin content that may be linked to a decline in red blood cell (RBC) count, which in turn suggests induction of anemic condition owing to toxic effects. Reduced levels of mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in rats (Ishinishi et al. 1988) and a rise of 3-5% in carboxyhemoglobin saturation in rats (Karagianes et al. 1981) were the only other criteria of erythrocyte status and associated events. An equilibrium between erythropoiesis in bone marrow and its catabolism in the spleen and liver is necessary for blood homeostasis. The erythropoiesis and total erythrocyte count of birds are disrupted by both the kinds of foods they consume and the stressors they are exposed to. The hematological effects of an artificial *Ascaridia galli* egg infection in broiler chickens were investigated by Arunachalam et al. (2003). All infected groups of cockerel birds showed a statistically significant decrease in Hb gm%, which might explain their lower average body weight and altered foraging behavior. This conclusion is consistent with what Pavitrakar et al. (2005) found when they studied the impact of herbal therapy on certain hemato- biochemical parameters in cockerels with induced ochratoxicosis. The cockerels' renal function was impaired, resulting in lower hemoglobin and packed cell volume. Cockerel birds exposed to the agent had higher neutrophil, lymphocyte, monocyte, eosinophil, and basophil percentage levels. However, when compared to the control group, there was no significant difference.

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