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RESEARCH ARTICLE **OJMR0301001-263**

EVALUATION OF POULTRY HOUSE DUST AND ITS EFFECT ON THE BIOCHEMICAL, HAEMATOLOGICAL AND HISTOLOGICAL PARAMETERS OF WISTAR RATS IN EDO STATE

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ABSTRACT

Poultry farmers and birds are exposed to poultry dust which is a byproduct of commercial poultry production. It increases the chances of developing serious respiratory conditions. Adult male Wistar rats were used in this study due to their stable hormonal status to investigate the effect of poultry dust (PM_{10}) on the biochemical, haematological and histopathological parameters of the animals. The Wistar rats were exposed to dust samples collected from 9 selected poultry farms in Edo State. Indoor concentration of dust samples was determined monthly using a Casella Cel 712 micro dust pro air sampler from December 2016 to November 2017. Wistar rats were exposed to sieved poultry dust obtained with the aid of a vacuum cleaner equipped with a 25- m mesh paper dust bag. Blood samples and organs were obtained from sacrificed Wistar rats for biochemical, haematological and histopathological studies. Poultry dust (PM_{10}) concentration were above recommended limits (0.15mg/m^3) of the United State Environmental Protection agency (USEPA). There was significant reduction in RBC, Hb, PCV, MCV, monocytes and lymphocytes count in dust-exposed Wistar rats compared to unexposed ones. There was also evidence of histopathology attributed to exposure. This study therefore revealed high dust concentration that can result to a number of health impacts in mammals within the poultry environment.

Keywords: *Poultry Dust, Wistar Rat, Biochemistry, Haematology, Histopathology*

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INTRODUCTION

In poultry houses, airborne dust concentrations are usually high and long-term exposure to excessive quantities of dust could cause health problems for poultry employees and birds who live in the facility 24 hours a day (Pickrell, 1991). Majority of poultry dust is made up of manure, feed, feathers, dander, litter and a variety of biological pollutants (Dauda *et al*., 2016).

Dust contains inorganic particles from building materials such as concrete and insulation used in barn construction. Because of their irregular shape, these particles provide a vast surface area for adhering bacteria (or their components), virus particles and mold (Zhang *et al*., 2019). The size of the dust particle is essential because larger dust particles tend to settle from the air near their source. Smaller dust particles stay in the air for a longer period of time, increasing the likelihood of inhalation. Airborne dust levels can be high enough in some poultry barns to create a visual haze, making it impossible to see from one end of the facility to the other in long barns (Pickrell, 1991).

Inhalable and respirable dust are the two types of dust. Particles that are small enough to be breathed but get trapped on the upper respiratory tract mucosa such as the mucosa enclosing the nasal turbinates, trachea or large bronchi are known as inhalable particles. This includes particles with a diameter of less than 10 microns (m) for humans (PM_{10}). Respirable particles in humans are typically smaller than 2.5 µm in diameter ($PM_{2.5}$) and may travel deep into the lungs all the way into the gas exchange surface (alveoli). Particles with a diameter of 2.5 m can get deep into the parabronchi and air capillaries in poultry, which are the structures where gas exchange occurs (Ilahi, 2012).

Studies in epidemiology have indicated that airborne participate matter (PM) with mass median aerodynamic diameter <10µ.m (PM_{10}) has been linked to an increase in respiratory illness. However, there is a growing consensus that particles larger than 2.5 microns ($PM_{2.5}$), including many in the ultra-fine (0.1 micron) size range cause more harm."In animal studies, particle mass, composition, size and number determine the toxicity of inhaled PM." (Smith *et al.*, 2003).

According to the USEPA, particulate matter in the air is a complex blend of organic acid and 51 inorganic components (Smith *et al*, 2003). Smith *et al*. (2003) investigated the physicochemical properties of concentrated particles in California's central valley between the fall of 2000 and the winter of 2001. Total suspended particles are found in larger numbers in poultry and swine buildings than in human buildings, implying that poultry and swine buildings are dustier than human buildings (Pickrell, 1991). According to the researchers, total dust levels ranged from 0.02 mg/m^3 in an unused area of a facility that held 7-day-old birds with new bedding or litter to 11 mg/m^3 in the front half of a confinement building carrying 30-day-old birds. Each dwelling's dust readings revealed a small but consistent ratio of respirable to total dust of roughly 5%.

Animal-derived particles (manure particles) are significantly smaller than other particles and can be deposited deep in the lungs. Particle clearance by macrophages is inhibited with enough deep depositions, increasing the effect of exposure. Both animal and human health are harmed as a result of such exposure. (Pickerell, 1991).

Workers on poultry farms do develop respiratory symptoms as well as immunological abnormalities. Inhaling organic dust (feces, feathers, etc.) for an extended period of time might cause hypersensitive lung illnesses (extrinsic allergic alveolitis). Coughing with sputum and wheeze have been reported as symptoms (Reynolds *et al*., 1993).

Psittacosis, aspergillosis, and histoplasmosis are among the infectious respiratory diseases linked to dust exposure in the poutry industry" (Farooq *et al*., 2002). The objective of this research is to evaluate Indoor dust concentration in poultry farms in Edo State, Nigeria and the effect of the poultry dust (PM_{10}) on the biochemical, haematological and histopathological parameters of Wistar rats.

MATERIALS AND METHODS

STUDY AREA/ STUDY LOCATIONS

The Data for this study were collected from nine selected poultry farms in Edo State. The State is divided into three senatorial districts, namely: Edo Central, Edo North and Edo South and lies between longitudes 05º 041 E and 06º 431E and latitudes 05º 441N and 07º 341N of the equator. Dust concentrations and samples were collected from selected poultry farms in Auchi, Ekpoma and Benin City.

SAMPLE COLLECTION

Measurement of Dust Concentration.

The concentration of dust of aerodynamic diameter of <10mn was determined electronically with the aid of a direct reading active personal sampler, Casella cell dust (Environmental Device co-operation, U.S.A). An active sampler uses a pump and a power source to move air through a collector (WHO, 2000). The sampler has a sampling flow rate of 1.0 l/mins and the instrument software allows direct reading of dust concentration. The sampler was placed 1.5m above the floor, the device switched on and dust concentration determined after 1 minute and measurements were taken on monthly basis in each of the poultry houses investigated. The results were expressed in $mg/m^{3.}$

Poultry dust sampling and animal treatment: The poultry dust used for this experiment was obtained from the air present at selected poultry farms across Edo State. To obtain representative dust samples in the study area of the selected poultry farms, (Benin City, Ekpoma, and Auchi), dust samples were collected in the morning between 9am and 11am from the three sampling points from poultry farms. A vacuum cleaner (Morphy RichardsTM Model-37164) with a 25-m mesh paper dust bag was used to collect the dust samples (Allied Filter Fabrics, Sydney, Australia). The same vacuum cleaner was used to collect all of the samples from the Poultry Farms. The dust bags were removed, sealed, and the suction hose was cleaned by turning it on and drawing only air for 10 minutes between samplings after each poultry was sampled. For the exposure, a large amount of dust was gathered. Many coarse particles such as sawdust and bigger particles such as dust mites were found in the samples. To eliminate the coarse particles, the samples were sieved through a 150 m meshed laboratory sieve. Before exposing Wister rats to these, they were well mixed in a plastic container and sieved to 2.0–3.0 m diameters. Dust dispersal and suspension in the air was achieved with the aid of a rechargeable fan. The reactions of the rats to poultry dust during exposure in the cages were observed and recorded (Eteng *et al*., 2018).

Animal breeding: The experiment involved thirty (30) adults male wistar rats weighing between 100 and 160g. Male Wistar rats were used in this study due to their stable hormonal status. The rats were purchased from Animal House, Department of Anatomy, College of Medical Sciences, University of Benin and housed in the Animal house, Faculty

of Agriculture, University of Benin, Benin City. The animals were maintained in a 27±1.5°C temperature, 60%±5% relative humidity and 12 hrs day and night cycle and were managed in accordance with laboratory animal standard protocols that had been implemented and cited by Ozolua *et al.* (2009). The rats were kept to acclimatize for a period of seven days before commencement of the experiment. They were fed twice daily with grower chicken feed (corn mash). Clean water was provided in plastic water bottles with plastic nozzles. The rats were divided into five groups (A, B, C, D, and E), each with six (6) rats. Each group was housed in a wooding cage with an aluminum net covering for proper ventilation and the cages were kept clean, dry and warm by covering the floor with wood chips.

Wistar rats in group A were not exposed to poultry dust which served as control while groups B, C, D and E rats were exposed for 3 hours per day for a period 6 weeks Smith *et al*. (2003).

Animal sacrifice: Twenty four hours after the last day of exposure, the rats were weighed and anaesthetized by intraperitonaneal injection at a dosage of 100mg/kg body weight. The rats from each group were sacrificed by the chloroform inhalation method. Blood samples were collected via the heart from each rat into EDTA bottles and taken for laboratory analysis haematological studies. The rats were exsanguinated via the abdominal aorta. Lungs, Liver, Kidney and Spleen were harvested for histological studies.

Haematological Parameters Determination

The Department of Haematology at the University of Benin Teaching Hospital used an automated Sysmex XE-5000 haematology analyzer from Japan to carry out the analysis. The plasma biochemical analysis was carried out using the COBAS Integra 311 England automated analyzer. Hematocrit (HCT), total white blood cell (WBC) count, packed cell volume (PCV), hemoglobin (HGB), red blood cell (RBC), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin (MCHC) were determined. Others were the percentage of lymphocytes, the amount of lymphocytes, and the number of platelets (PLT). Clinical laboratory findings were used to determine the liver profile (total protein, albumin, creatinine, and alkaline phosphatase). Three enzymes that help the body break down proteins include phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). The presence of urea was also determined (Igbinaduwa and Aikpitanyi-Iduitua, 2016).

Histopathological and morphological studies

Under chloroform anaesthesia, organs (liver, kidneys, lungs, and spleen) were removed and fixed in neutral buffered formalin. To properly dehydrate the fixed organs, absolute ethanol was utilized first, followed by 96% ethanol, 70% ethanol and lastly distilled water.

A 4m segment was cut and stained with haematoxylin-eosin dye in each case. The stained tissues were examined under an optical photomicroscope (Leica MC170 HD, LeicaBiosystems, Germany) For all animal groups, detailed microscopical studies of organ sections were performed and Photomicrographs were taken at two magnification levels of 100 and 400 (Ogbera *et al.*, 2007). A histology specialist performed the analysis and interpretation of the results.

Statistical Analyses

The results of this study were given as mean SEM (standard error of the mean) or percentages. To test for statistical difference between the treatment and control groups, the t-test statistics were utilized. The statistical package SPSS version 21.0 for window evaluation version was utilized in the majority of cases for data analysis. Measures of central tendency (mean standard deviation) were used to assess values in triplicates. To compare various variables, a one-way ANOVA was done and the Duncan multiple range test was used to test if there was a significant difference in mean. Statistical significance was defined as a P-value of less than 0.05 (Ogbeibu, 2015).

RESULTS

The dust concentration of the sampled poultry farms revealed that the poultry dust presented in these environments were above the reference hourly permissible limit of the United States Environmental Protection Agency (USEPA). The highest dust concentration was recorded in the month of December 2016 in Ekpoma (Table 1). This period is usually characterized by dusty winds and atmospheric pollution. The lowest concentration was recorded in the month of June 2017 in Benin City. This period was characterized with high amounts of rain fall which resulted in improved air quality thereby decreasing the dust concentration.

Table 1: Dust concentration of poultry farms in Edo State (mg/m^3)

	Benin Dust (mg/m^3)	**Ekpoma** Dust (mg/m^3)	**Auchi** Dust (mg/m^3)
US EPA limits	0.15	0.15	0.15
Dec. 2016	1.06 ± 0.13^{f}	1.27 ± 0.20^{e}	1.16 ± 0.25
Jan. 2017	0.53 ± 0.09^{bcd}	0.75 ± 0.17^{abcd}	0.65 ± 0.13
Feb. 2017	0.53 ± 0.08^{bcd}	0.78 ± 0.18^{abcd}	0.62 ± 0.13
Mar. 2017	0.65 ± 0.09^{cde}	0.67 ± 0.15^{abcd}	0.65 ± 0.14
Apr. 2017	0.43 ± 0.07^{abc}	0.56 ± 0.12^{abc}	0.44 ± 0.08
May 2017	0.45 ± 0.07^{abc}	0.63 ± 0.11^{abc}	0.50 ± 0.09
June 2017	0.24 ± 0.02^{a}	0.50 ± 0.79^{ab}	0.31 ± 0.05
July 2017	0.27 ± 0.03^{a}	0.43 ± 0.08^{a}	0.29 ± 0.06
Aug. 2017	0.29 ± 0.04^{ab}	0.35 ± 0.05^{a}	0.28 ± 0.06
Sept. 2017	0.72 ± 0.11^{de}	0.93 ± 0.17^{bcde}	0.77 ± 0.18
Oct. 2017	0.79 ± 0.09^{e}	1.01 ± 0.16^{cde}	0.88 ± 0.19
Nov. 2017	0.84 ± 0.09^{ef}	1.10 ± 0.16^{de}	0.79 ± 0.16
p- value	<0.001	<0.001	<0.001

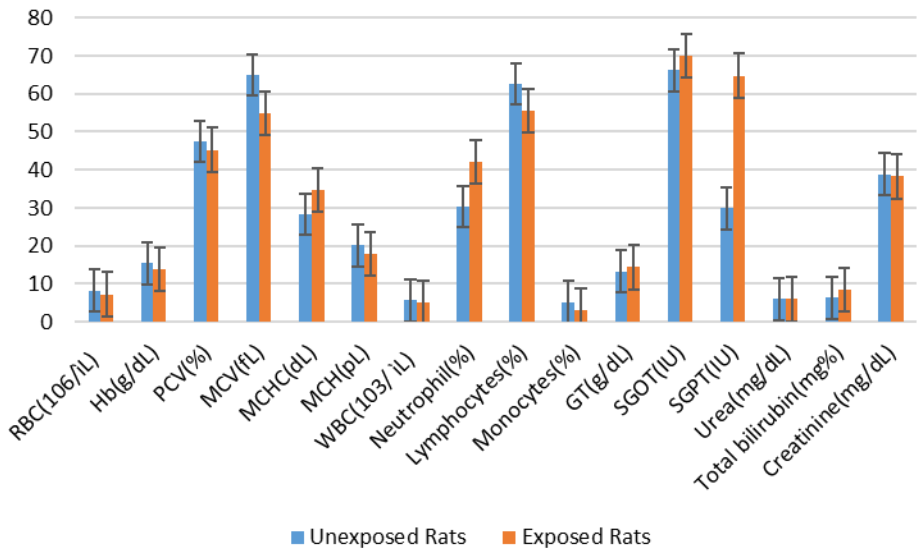

Figure 1: Haematological and Blood Chemistry of Wister Exposed Rats to Poultry Dust

Key.

MCH -Mean Corpuscular Heamoglobin

MCV- Mean Cell Volume

GT-Gamma Glutamyl Transferase

RBC -Red Blood Count

SGOT- Serum Glutamic Oxaloacetic Transaminase

HB-Heamoglobin

SGPT-Serum Glutamic Pyruvic Transaminase

WBC- White Blood Count

MCHC-Mean Corpuscular Hemoglobin Concentration

The results of the analyzed haematological and biochemical parameters of the rats exposed to poultry dust are presented in Figure 1. The Wistar rats that were used in this study as subjects to model the potential effects of dust from poultry houses and the effects on the hematobiochemical parameters in poultry workers and possibly the poultry birds in the absence of a comprehensive hygienic state. Results presented in the figure showed that there was statistically significant percentage increase in neutrophil (40.3%), SGOT (5.8%), SGPT (117%) total bilirubin (32%) levels as compared to control subjects. The haemological analysis revealed that 12.68%, 4.6%, 10.1%, 11.34% and 43.8% significant percentage decrease in red blood cells count (RBC), packed cell volume (PCV), white blood cell count (WBC), lymphocytes and monocytes respectively were also recorded in dust exposed rats.

Histopathological examination of lungs, kidney, liver and spleen of dust exposed Wistar rats showed significant changes in tissue morphology. Degeneration of the lung tissues and intra-alveolar thickening were observed (Plates 1 - 4).

The Wistar rats exposed to poultry dust also displayed ocular and nasal discomfort, restlessness for the first 40 mins, which was followed by calmness while the eyes remained closed and nostrils hidden. The display of ocular and nasal discomfort resumed whenever the exposure was being done. Nephropathy was reported (Plates 5 – 8). Tubular degeneration of kidney tissues accompanied with swollen glomeruli was reported and this advanced to

necrosis and oedema. Liver cells of dust exposed rats presented hemorrhagic features with tissue disintegration (Plate 9 and 10). The spleen cells also showed some mild distortions (Plates 11 and 12).

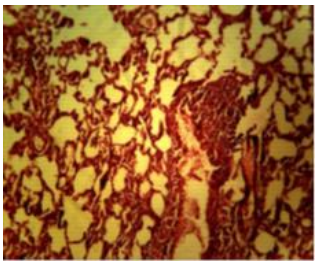

Plate.1:. Photomicrograph of a Wistar Rat's Normal Lung X100, H&E.

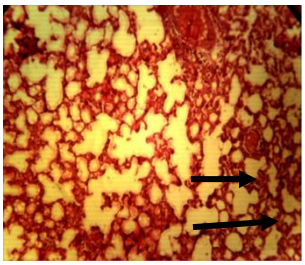

Plate. 2: A photomicrograph of a Wistar Rat's lung reveals early degeneration. X100, H&E.

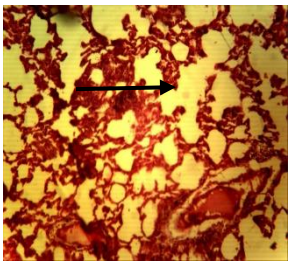

Plate 3: A photomicrograph of a section in the lung of a rat showing early degeneration and vacuolation of cells. H&E., Mic. Mag. X 100.

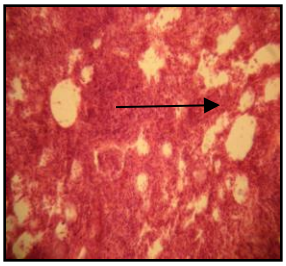

Plate 4: Photomicrograph of an exposed wistar rat showing congestion, narrowing of air spaces and interalveolar thickening. (Transverse section, H&E stain, x400).

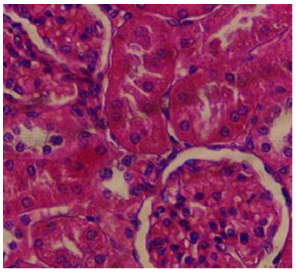

Plate 5:
Photomicrograph of the kidney of unexposed wistar rat (H&E, X400).

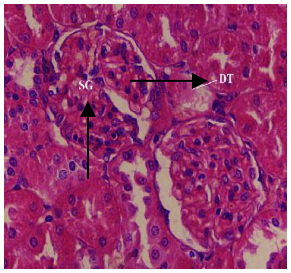

Plate 6:
Photomicrograph of kidney of rats showing tubular degeneration (DT) and swollen glomeruli (SG). H&E, X400.

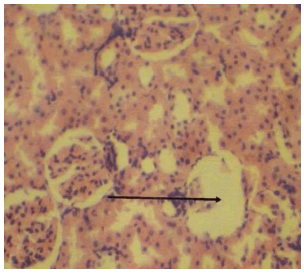

Plate 7:
Photomicrograph of kidney of wistar rats showing hypertrophy of Bowman's infiltrates of inflammatory cells and severe loss of tubules H&E, X100.

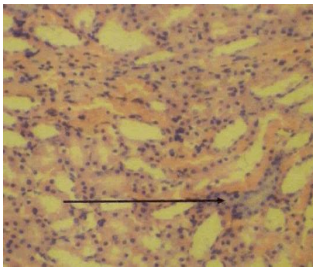

Plate 8:
Photomicrograph of kidney of wistar rat showing distortion of interstitial tissue architecture, signs of necrosis, infiltration of inflammatory cells and oodema X100

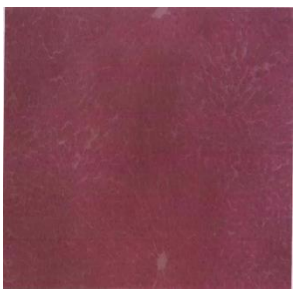

Plate 9: Photomicrograph of a normal liver of wistar rat (Control) (H & E stain x 100)

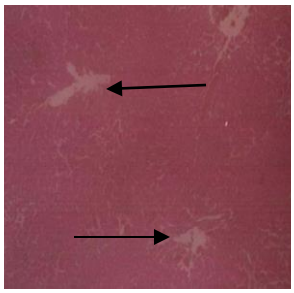

Plate 10: Photomicrograph of liver of wistar rat. The circled areas show heamorrhagic features with minor disintegration of hepatic cells (H & E stain x 100)

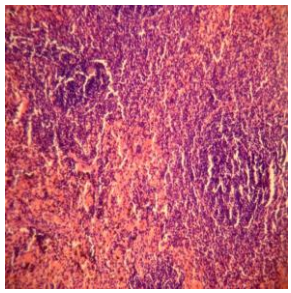

Plate 11: Photomicrograph of spleen on an unexposed wistar rat x100).

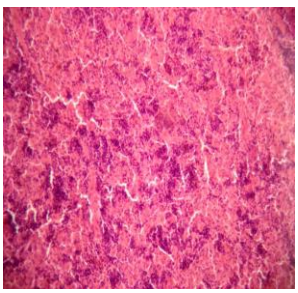

Plate 12: Photomicrograph of spleen of rat exposed to poultry dust (Transverse section, H&E stain, x100)

DISCUSSION

Results from poultry dust exposure showed significant reduction in red blood cells counts (RBC) and packed cell volume (PCV) percentage similar to reports of Dauda *et al*. (2016); Eteng *et al. (*2018) and Ahmad and Akhter (2018). Decrease in RBC and PCV may be manifestation of deleterious effect of PM_{10} on the bone marrow with resultant anaemia. There was however no significant difference between haemoglobin concentrations, MCV and MCHC in control and dust exposed rats. Decrease PCV leads to impairment of oxygen transport resulting in tissue hypoxia, which may manifest as respiratory illnesses (Dauda *et al.,* 2016).

There was significant reduction in white blood cells counts during this study contrary to reports by Eteng *et al.*, 2018 and Dauda *et al.*, 2016 who recorded significant increase in WBC in their separate studies. These conflicting findings may be due to the fact that the dust in this study was from organic source. However further studies should be conducted that will subject rats to dust from inorganic and organic sources where all other conditions will be kept similar. The observed difference may also be attributed to the fact that exposure in this study was for a longer duration than those of previous authors, therefore there is the possibility of the white blood cells being adapted to the condition after previously being mobilized. Neutrophil levels were however significantly raised in dust exposed rats similar to results obtained by previous authors (Dauda *et al.*, 2018). This is an indication that the dust in this study has high bioaerosol load and neutrophils are responsible for phagocytic actions and engulf cell debris.

The mean SGPT, total bilirubin and SGOT levels were significantly higher in dust exposed subjects and this is in agreement with previous work by Ilahi *et al*. (2012). SGPT and bilirubin are localized in the liver and high amount in the serum is indicative of damages to hepatic tissues. Dust exposure assessment during this study showed significant potential effect of poultry dust on red blood cells, P.C.V, SGPT as well as bilirubin levels.

Degenerative tendencies observed in the lung as a result of dust exposure is a clear manifestation of damages to the lung tissues thus conforming to studies of Zhang *et al.,* 2018 who reported pneumoconiosis and pulmonary fibrosis in tracheal and non-tracheal exposed rats respectively. Histopathological differences observed in dust exposed rats in this study is similar to reports of Ilahi *et al*. (2012), who suggested that high levels of SGPT and bilirubin levels are clear manifestations of damaged liver cells. With the ever-increasing global population, there is resultant increase demand for food, of which poultry meat is not an exception. There has been great deal of researches into the possible effect of the proliferation of poultry farms to meet human demand for poultry products and its implication on human health as well as the environment. Investigation into these effects has included airborne pollution potentials of poultry facilities.

Histopathology results from dust exposed rats also showed significant damages to lungs, liver and kidney while the spleen cells showed mild distortions.

Further studies should investigate the levels of poultry outdoor airborne pollutants at different distances away from the facilities to determine the potential risk associated with poultry pollutants with distance from the facility.

CONCLUSION

The study revealed that poultry dust posed significant threat to the haematological and histological functions of the experimental animals. It is obvious that extended exposure to poultry dust is a major occupational health risk and workers should be required to wear nasal masks when working in poultry confinement facilities. It is also suggested that a regulatory code of occupational health and safety surveillance be devised for the protection of poultry employees and poultry birds.

ETHICAL CONSIDERATIONS

The authors followed the ethical guiding principle controlling the use of laboratory animals as specified by the Committee on Ethics for Medical and Scientific Research, University of Benin, Nigeria. The Canadian Council on Animal Care Guidelines and Protocol Review, which are internationally recognized requirements for the use and care of laboratory animals, were also followed.

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