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Investigating Endosulfan Consequences: Resistance, Immunology, and Teratogenic Manifestations in Layers and Their Offspring

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Abstract

Endosulfan, a common organochlorine pesticide, has recently been focus of significant research owing to its negative effects on the surroundings and on living species. The study aimed to assess to immunological effects of limited oral administration of endosulfan insecticide in white leghorn layer chickens. Twenty white leghorn birds were given endosulfan in drinking water at a dosage of 40 ppm/bird/day for three months, which is a detectable effect level dose. Immunological competence was evaluated in layer birds every 16 days and in chicks hatched from endosulfan-exposed birds on a monthly basis. Various immunological, biochemical, and teratological parameters were measured. The study recognizes the significant of investigating endosulfanuses an ANOVA test to resistance, immunology, and their offspring. The white leghorn bird dataset are collected. The quantity of oxalate in eggs improved considerably for both the White Leghorn (p0.07) and Dwarf Layer (p0.004) species after Marigold Flower Extract (MFE), rising to 212.60 μg/egg and 166.80 μg/egg, respectively. The breeds fed the Control (CON) and an MFE diet was not able to exhibit significant variations in the quantity of carotenoid in their eggs.The goal of this research is to clarify the complex effect of endosulfan exposure on both layers and their offspring to inform health and environmental policy.Tests for immune competence, such as the enzyme linked immunosorbent assay (ELISA) and the lymph cell stimulating test indicated that endosulfan treated birds showed decreased immunity. Interestingly, to significant differences were observed inthe avian endosulfan treatment's effect on the immunological competence of the offspring as compared to the control group. The study suggests that limited oral exposure to endosulfan induces hemo biochemical changes associated with alterations in the immunological profile of layer chickens. The results therefore support the use of endosulfan insecticide in chicken sheds selectively to reduce any effects on the immune system.

Keywords: Endosulfan, Environmental Impact, Immunology, layers, white leghorn bird

INTRODUCTION

Since the 1950s, endosulfan, a chlorinated hydrocarbon insecticide, has been used globally, but many nations have banned or restricted its use due to its persistent nature (1). A multitude of data points to the harmful effects of endosulfan on non-target animals. Considering the broad-spectrum action, endosulfan is utilized in insect control procedures; however, non-target species might be at risk from its persistent leftovers (2-3). Layers, referring to female poultry and their offspring are vulnerable due to their close proximity to the agricultural ecosystems where endosulfan is employed (4). To investigate the effects of endosulfan exposure on layers and their progeny, with a particular emphasis on three crucial aspects: the development of target pest resistance, the immune responses in layers and possible teratogenic effects in the progeny (5). Layer birds could have negative effects on their immune competence from the broad-spectrum pesticide endosulfan (6). The induction of efficient immune responses in birds

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can be compromised due to hypertension connected to endosulfan exposure. Layer bird health and production could be compromised by the pesticide's harmful effects on the immune system, upsetting the delicate balance (7). To protect the birds' immune systems and guarantee the production of healthy, safe eggs for human consumption, it is essential that endosulfan exposure in poultry farming be minimized and monitored (8). The birds' general well-being and health are threatened by these immunological alterations, which can make them more vulnerable to illness (9). Ensuring sustainable poultry farming operations and reducing the detrimental effects of endosulfan on the immune systems of layer birds requires stringent regulatory measures and monitoring (10).

Resistance in Layers

The observed prevalence of endosulfan resistance in pest populations has been connected to the prolonged and widespread usage of this pesticide. The potential for layers subjected to repeated exposure to acquire resistance (11). Examining the genetic and physiological processes that could lead to resistance of these birds is required to comprehend the lasting effects of endosulfan consumption.

Immunological Responses

The immune systems of layers, hatchability and egg quality are intimately connected to each other. Endosulfan's immunotoxicity raises concerns regarding potential effects on layers' immune responses (12). To find out the way long-term endosulfan exposure impacts individuals' immune systems and if this can make them less resistant to infections and disease. To assess the broader implications for poultry health, it is important to recognize the generational effects on the immune system of offspring (13). The acceptance, immune reactions and teratogenic effects of endosulfan exposure in layers and their offspring are examined in this work (14). Analyzing the precise changes in immunological markers, such as immune cell activity and antibody production, is crucial for assessing the general well-being and state of health of individuals (15). More information on the wider impacts of endosulfan exposure on poultry health could be acquired from the effects on the immune system of offspring. The primary goal of study is to describe the effects of endosulfan in layers and their progeny is to thoroughly evaluate resistance patterns, immunological effects along with teratogenic symptoms.

The study (16) explained the impact of a low dosage of endosulfan sulfate (ES), a bio persistent contaminants identified in both ambient and human specimens, yet an important component of the insecticide endosulfan, on the development of metabolic disorders as well as obesity. From intrauterine day 6 to postnatal day 21 (immediate), ES was administered to pregnant CD-1 mice. Male pups from exposed females were fed either a high-fat or low-fat diet (HFD) after transition and after an extra 12 weeks, they were evaluated. A single set of male pups got ES oxidation of fatty acids and disruption of the makeup of the gut micro-biota, ES therapy prevented weight gain produced by HFD, according to analysis of gene expression, metabolic profile and intestinal micro-biota. The article (17) described the effects of co administration of methanolic and aqueous extracts of the entire plant of Catharanthus roseus on the development of drosophila melanogaster, along with the alterations that the amount of endosulfan and its beta and alpha isomers of the accomplished. To ascertain the quantity of alpha beta and endosulfan EC70 and provide it with an aqueous and methanol extracted $1/60^{th}$ EC30 level. To assessed the ameliorative effects of these concentrations by imaging live neurons and confirming the life phase activities. Subsequently came to the view that the aqueous and methanolic extracts equally increased the test organism's survival rate and inhibited the harmful effects of endosulfan and its isomers. The research (18) described that birds worldwide has elevated concentrations of white leghorn birds. According to laboratory and epidemiological research, birds interfere with the endocrine, immunological as well as neurological systems, as well as reproduction, development and growth, in birds, causing a wide range of adverse consequences. Different findings indicate that it might be several effects of exposure on the physiological parameters in birds, such as maturity, gender and chemical category, along with a variety of biological

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processes and particular changes. The study (19) used the zebrafish model of animals to assess the developmental toxicity of endosulfan at higher ambient temperature. Zebrafish embryos at different developmental stages were cultured in E3 medium, exposed to endosulfan and kept at two specific temperatures (23 °C and an enhanced 45°C) while that was observed under a microscope. Endosulfan therapy under the high temperature conditions had a synergistic effect on the stress implicated genes hsp70, p16 regulators. In zebrafish eggs, the increased ambient temperature increased endosulfan's toxic effects on development.

MATERIAL AND METHODS

Data analysis

200 birds were selected at random and had their bodies measured for the present analysis were collected, with data collected from both genders combined. The current research made use of synthetic White Leghorn strain records kept at chicken reproduction property; this strain has been at the farm since 1977 and it was created as a consequence of intentional breeding conducted over several generations in the White Leghorn population. To enhance the genetics of the breed, one necessity is to integrate selection based on 40 week egg produced and egg size (20).

EXPERIMENTAL DESIGN

All of the birds in this study underwent a strict protocol to ensure the reliability and uniformity of the experimental environment. It were kept in a standardized deep bedding system, fed and cared for consistently. Entire disease control protocols were put in place to keep study participants' health at its best. The chosen birds were given an intraocular Ranikhet vaccine F1 strain as a primary and booster dose to strengthen immunity. Furthermore, the progeny of layer birds exposed to endosulfan underwent extensive immune competence testing in an effort to assess the possible effects of endosulfan on the immune systems and general health of the following generation. The validity and applicability of the study findings are improved by this systematic approach. To evaluate the effect of endosulfan exposure on the treated group relative to the control group, the experiment was split into two separate phases. The original solution was diluted 1:9 with water to create an endosulfan solution in the first section. The birds in the treatment group were given this resultant solution, which had a concentration of 30 ppm based on persistent toxicology testing, every day mixed into their drinking water. To ensure a baseline unaffected by endosulfan, the control group was given normal water in the meantime. A comprehensive analysis of the particular effects of endosulfan exposure on the experimental subjects was made possible by this methodical division.

Endosulfan impact on layer birds

In the initial phase of the experiment, 30 birds were randomly assigned to two groups: the control (C1) group and the endosulfan-treated group (C2). Each group consisted of 20 birds with 18 hens and 2 hens. Blood samples were collected from the wing vein at 40 day intervals over a 90-day period for both groups. The first blood samples are stored in heparinized vials (15-20 ml) to examine biochemical measures such as albumin, globulin, alpha globulin and total body protein. Simultaneously, to conduct immunological tests and assess antibody titers using an enzyme-linked immunosorbent assay (ELISA) with the protein of the Ranikhet virus, a subsequent batch of fluid samples were obtained and placed in sterile vials. This systematic approach allowed for a detailed assessment of both biochemical and immunological parameters in response to endosulfan exposure. Immunology studies are following as,

- Con-A and LPS mitochondria were used for lymphocytes blast genesis.
- Test for total protein, albumin, globulin and gamma globulin is biochemical.

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- Ranikhet sickness viral antigen ELISA antibody titer.

Endosulfans effect on feather bird chicks

The experiment's second portion examined the immunological competence of endosulfan-fed chicks. Each batch of chicks was separated into three categories: those from birds treated after 1 month, 2 months and 3 months of endosulfan therapy. Chicks from both groups were managed for one month. At a month's time of age, specimens of blood were extracted through the heart and divided into two parts: heparinized vials for hematological and immunological analyses and sterilized vials for serum collection. Endosulfan's teratogenic impact was assessed by studying hematological/immunological and biochemical markers in chicks and their parents. Endosulfan was not administered to any chicks.

TERATOGENIC LAYERS AND OFFSPRING

The teratogenic potential of endosulfan its capacity to disrupt development has been well documented in a number of animals. To determine the precise teratogenic effects layers and their progeny experience after being exposed to endosulfan. The amount of this pesticide's harm, an analysis of the reproductive organs, embryonic development and overall reproductive performance is necessary. Teratogenicity is ability to produce defects in development, is an essential concern for both the individual and the generation that follows. By a detailed examination of reproductive organs result in total reproductive success and embryonic development, the research attempts to identify the precise effects of endosulfan on the anatomical and functional characteristics of birds. Recognizing these teratogenic expressions is essential in evaluating the possible hazards to the health of birds and the wider consequences for the food chain.

STATISTICAL ANALYSIS

Statistical techniques include Analysis of Variance (ANOVA) is utilized to examine the resistance, immunology and teratogenic symptoms in layers and their progeny. ANOVA is a potent statistical technique that allows for comparison means across many groups, which makes it appropriate for concurrently examining numerous parameters. Important factors influencing the health and production of layers and their offspring in the context of chicken farming include immunological responses, disease resistance and the emergence of teratogenic symptoms. ANOVA tests allow researchers to investigate the effects of many variables, including as nutrition, environment and genetics, on its characteristics. ANOVA provides easier to identify significant variations in resistance levels, immunological markers, or teratogenic effects across groups of layers or their offspring. This information is essential for designing effective breeding programs, enhancing management strategies and coming up with measures to enhance the overall health and welfare of chicken populations. ANOVA is a useful tool for poultry scientists studying the intricate interactions between immunity, teratogenic manifestations and resistance in layers and their progeny, which helps to improve the health and productivity of birds as shown in Equation (1-3).

$$PT_{between}/QT_{error} \quad (1)$$

Where,

$$PT_{between} = \frac{\sum_{i=1}^{k} k(\bar{X}_i - \bar{X})^2}{O-1} \quad (2)$$

$$PT_{error} = \frac{\sum_{i=0}^{N} \sum_{j=1}^{j} (x_{ji} - y_j)^2}{N-L} \quad (3)$$

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The definition of the Welch test statistic is expressed in equation (4):

$$Z=\frac{\sum_{i=1}^{k} z_j\left[(x_i-\tilde{x})^2/(l-1)\right]}{1+\frac{2(k-2)}{k^2-1}\sum_{i=1}^{k}\left[\left(1-y_j/u\right)^2/(m_i-1)\right]} \quad (4)$$

Where $m_i=\frac{m}{s_1^2}$, U= $\sum_{i=1}^{k} y_j$, and $Y=\frac{1}{u}\sum_{i=1}^{k} m_i\, y_j$ is defined in equation (5):

$$Y=\frac{k^2-1}{3\sum_{i=1}^{k}\left[\left(1-y_j/u\right)^2/(m_i-1)\right]} \quad (5)$$

Equation (6) illustrates the definition of the Brown-Forsythe test statistic.

$$G^*=\frac{\sum_{i=1}^{k} R_i(\bar{x}_i-\bar{x})^2}{\sum_{i=1}^{k}(1-m_i/M)T_j^2} \quad (6)$$

When M is true, the degree of freedom t in a central G distributed with levels of freedom $k-1$ and $G*$ is appropriately allocated as shown in Equation (7-9):

$$1/y=\sum_{i=1}^{k} c_i^2/(m_i-1), c_j=\frac{(1-m_i/m)T_i^2}{\sum_{i=1}^{h}(1-m_i/M)T_j^2} \quad (7)$$

The new g −value is determined as $g = 1-m_i$where t is the sample size.

$$Z=\left(R_{K-1m-k}\left(\frac{N-l}{M-1}\tilde{s}_c\left(\frac{H_1s_1^2}{C_1C_2,\ldots,C_{K-1}},\frac{H_2s_2^2}{C_1C_2,\ldots,C_{k-1}},\frac{H_3s_3^2}{(1-C_2)C_3,\ldots,C_{k-1}},\ldots\frac{H_1s_l^2}{(1-C_{l-1})}\right)\right)\right) \quad (8)$$

The estimation is determined using a $k-R, Z-H\,of$ distributed F-distribution and an independent Beta stochastic procedure.

$$B_l\sim Beta\left(\sum_{i=1}^{k}\frac{(m_i-1)}{2},\frac{m_{k+1}-1}{2}\right), m=1,2,\ldots,L-1 \quad (9)$$

The p-value is computed by integrating the predicted value for the Beta unknown variables in the p-value calculation.

RESULTS

Ratio of endosulfan

Endosulfan salt converting dropped at 90 days in all treatments for 50 birds; of Figure (1) and table (1) illustrate the endosulfan to standard therapy ratio, which is 30% in the standard therapy to 14.2, 7.6 and 2.6% in the 0.4, 0.6 and 2.0% biochar treatments, respectively. In addition, as the biochar content grew each treatment's percentage of endosulfan sulfates manufacture reduced with age.

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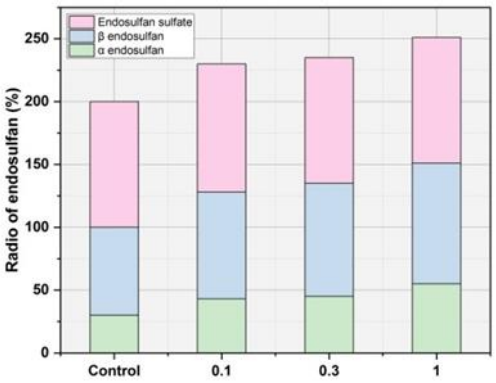

Figure (1). Biochar treated endosulfan levels at 90 days (source: author)

Table (1). Biochar-treated endosulfan levels at 90 days (source: author)

	Radio of endosulfan (%)		
	α endosulfan	**β endosulfan**	**Endosulfansulfate**
Control	30	70	100
0.1	43	85	102
0.3	45	90	100
1	55	96	100

Hemoglobin glycosylation

Blood glycosylated hemoglobin (GHb) levels among broiler chickens were exposed to the arsenic (3.2 ppm) in drinking water daily and given endosulfan 10 ppm through feed for sixty days. Arsenic (8.5%) and endosulfan (2.7%) raised the substance GHB level considerably when compared to the control, but the birds that were simultaneously treated to these compounds lacked any discernible changes illustrate the figure (2) and table (2). The GHb level increased much more in the birds treated with endosulfan than in the birds exposed concurrently.

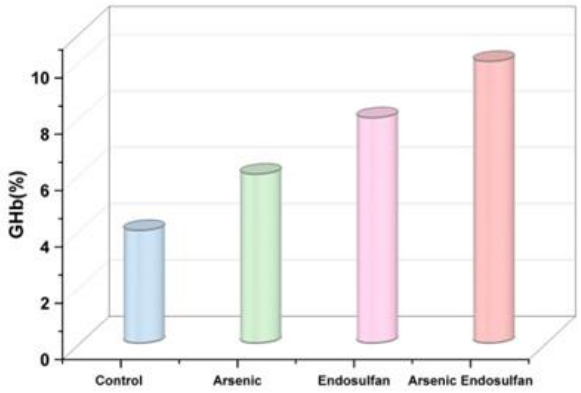

Figure (2). Glycosylated hemoglobin (GHb) in chicken blood (source: author)

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Table (2). Glycosylated hemoglobin (GHb) in chicken blood (source: author)

Levels	GHb (%)
Control	4
Arsenic	6
Endosulfan	8
Arsenic Endosulfan	10

Breeds' lutein deposition

The impact of feeding marigold flower extract (MFE) to various breeds on the accumulation of carotene in eggs is shown in figure (3) and table (3). Following MFE feeding, the amount of oxalate in eggs improved for Dwarf Layer (p 0.004) and White Leghorn (p 0.07) species, increasing to 166.80µg/egg to 144.50µg/egg and 212.60µg/egg, respectively. However, there were no discernible variation in the amount of carotenoid in the eggs between the breeds given control (CON) and MFE diets.

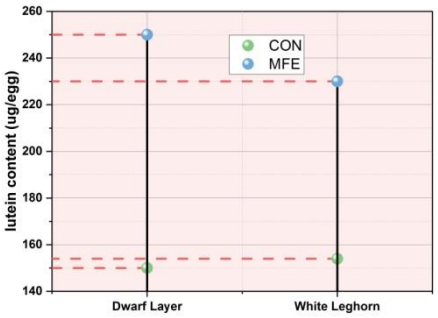

Figure (3). Egg lutein deposition by breed (source: author)

Table (3). Egg lutein deposition by breed (source: author)

Type	lutein content (ug/egg)	
	CON	MFE
Dwarf Layer	150	250
White Leghorn	154	230

CONCLUSION

This extensive investigation on the effects of endosulfan exposure on layer birds and their progeny used a strict experimental design while taking ethical issues into account. Understanding the metabolic, immunological and teratogenic impacts of endosulfan was made possible by the study. A thorough evaluation of the immediate and long-term effects was made possible by the methodical approach, which included strictly regulated exposure and diligent observation. In particular, the application of ANOVA in the statistical analyses of the research produced a strong framework for assessing immunology, resistance and teratogenic symptoms. We analyze ratio of endosulfan, hemoglobin glycosylation and breeds' lutein deposition**.** The chicken business benefits from these discoveries, which advance sustainable farming methods and well-informed breeding programmes that, prioritise environmental health and animal welfare. Future research on endosulfan effects will focus on resistance mechanisms, immunological effects and teratogenic effects in layers along with their progeny. To protect both human and poultry populations from the harmful consequences of endosulfan exposure, it is imperative that used to create efficient mitigation techniques.

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