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Comparing the Effects of Low and High Doses of Fosetyl Aluminum on Haematology and Serum Biochemistry in Japanese Quails *(Coturnix japonica)*

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ABSTRACT

Fosetyl- aluminium is an organophosphate which is extensively used to execute pest as well as act as a fungicides in agricultural fields to amplify the manufacture rate of crops along with fruits and vegetables and indirectly it have better manipulate on the non-target species particularly birds. Fosetyl-Aluminum is a systemic fungicide recommended for preventive applications on vegetables. It is practically not toxic to birds, aquatic organism and bees. It is proved through experimental study that submission of pesticides cause deleterious effects on organism's health throughout the world. In this experiment apparently healthy adult Japanese quail (*Coturnix japonica*) (n=30) were divided into three groups G0, G1 and G2; each group has 15 birds. For this study, quail of control group (G0) freed from dose while fosetyl aluminium is orally administrated to residual experimental group (G1) and (G2) at the rate of 1(ml/kg b.w) and 2(ml/kg b.w) for 10, 20 and 30 days of the experiment. Main intention of this experiment is to examine the toxic effect of concentration of fosetyl aluminium on haematology and serum biochemical changes of the quail (*coturnix japonica*). The blood and morbid tissues were collected at day 10, 20 and 30 of the experiment. The blood analysis indicated that Fosetyl- aluminium had a substantial impact on WBC, RBC, LYM, HCT, MCV, MCH, PLT, MPV, HGB, and MCHC counts in experimental groups. Moreover, serum urea, creatinine, bilirubin, ALT (SGPT) and ALP were also significantly affected. The present study revealed for the first time that fosetyl aluminium causes significant effects in birds in proportion to dose and duration.

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INTRODUCTION

Chemicals known as pesticides are employed in aquaculture and agriculture to eradicate or manage dangerous organisms that endanger human health or result in financial loss (Chen et al. 2013). Different pesticides can be used in aquaculture to prevent diseases in aquatic life(Aitken et al. 2016). Pesticides are poisonous compounds with specialized modes of action intended to kill organisms. The large range of pesticides on the market, with thousands of compounds being used (Bernhardt, Rosi and Gessner 2017). It is common knowledge that when pesticides are used in agriculture to eradicate weeds, pests, and fungal diseases, they also affect non-target species of plants and animals that are extremely susceptible to the harmful chemicals(Beketov et al. 2013, Habel, Samways and Schmitt 2019, Goulson and Nicholls 2016, Raven and Wagner 2021). Pesticides usually cause direct increases in the mortality or decreases in the fecundity of the target species, which lowers the quantity of organisms(Fleeger, Carman and Nisbet 2003, Sánchez-Bayo 2021). About two hundred thousand piles of chemical pesticides are used around the year worldwide with 24% being consumed in The United States of America and 45% in Europe (Abhilash and Singh 2009). Over the last decade, experiments conducted on red-legged partridges have revealed toxic effects of pesticides used as seeds treatments: the insecticide imidacloprid had a high acute toxicity(Lopez-Antia et al. 2015, Lopez-Antia et al. 2013), fungicides such as thiram, difenoconazole, flutriafol and tebuconazole negatively affected partridge's productivity(Lopez-Antia et al. 2021, Lopez-Antia et al. 2018, Lopez-Antia et al. 2015). The use of the most toxic active ingredients (imidacloprid and fipronil) in agriculture is currently banned in the European Union (EU). However, triazole fungicides, such as tebuconazole, flutriafol or difenoconazole, are still widely used as agricultural pesticides, particularly as seed treatments. These triazole fungicides can act as endocrine disruptors, altering the synthesis of reproductive hormones and thereby reducing the reproductive capacity of partridges(Lopez-Antia et al. 2021, Fernández-Vizcaíno et al. 2022). Despite the worldwide aim being driven toward more sustainable agriculture, pest management clearly depends on the usage of diverse kinds of pesticides. In Europe, around 380,000 tons of synthetic and inorganic pesticides are sold per year (average between 2011 and 2017 considering 28 European countries)(Chiaia-Hernandez et al. 2017, Fournier et al. 2020). Fosetyl-Al is a systematic fungicide that has been used to protect many fruits and vegetables against plant pathogens such as Phytophthora, Pythium, Plasmopara, Bremia spp. as well as bacteria such as Xanthomonas and Erwinia spp(Gormez et al. 2022). FOS that is sold under the trade name Aliette is an inorganic phosphorous systemic fungicide applied to control various plant pathogenic phycomycetes and ascomycetes, damping off and rotting of plant roots, stems, and fruit. This active substance is utilized for the prevention of crops and for the inhibition of fungal spore propagation and infiltration of pathogens into plants. It is applied as a plant dip treatment and a drench for transplants by incorporating it into the soil prior to planting and by applying it to foliage(Almeida et al. 2007). Fosetyl-aluminium (aluminium tris-O-ethyl phosphonate, fos-al), constitutes an alternative to phosphite, and it has been widely used in the management of diseases caused by Peronosporales (González et al. 2020), including some Phytophthora diseases of forest trees (Silva et al. 2016). fosetyl-aluminium is a systemic fungicide, which is widely used in agriculture to protect plants against various ascomycete and oomycete fungi as well as some bacteria which are pathogenic to plants in a wide variety of vegetables, ornamental crops, and fruits. . Fosetyl aluminium is highly soluble in water. It cannot persist in the soil and rapidly degrades into non-toxic compounds. It can cause severe eye irritation in humans; however, they are non-carcinogenic for laboratory animals, and they don't show any developmental toxicity, neurotoxicity, genotoxic potential or reproductive toxicity except when the doses are extremely high (Haq et al. 2023). Japanese quails (*Coturnix japonica*) belong to family Phasianidae and are being used as alternative research models to chickens (Farooq et al. 2022). They have a shorter life cycle, easily manageable and achieve sexual maturity around 7 to 8 weeks after hatching as reported by(Nasar 2016) and (Huss, Poynter and Lansford 2008). Quails serve as important model species for research on genetics, behavioural norms and avian reproduction(Nakane and Yoshimura 2014, Recoquillay et al. 2013). The ingestion of seeds treated with different triazole fungicides at commercial doses has adverse consequences on red-legged partridges(Fernández-Vizcaíno et al. 2020). Difenoconazole, a triazole fungicide, reduced egg length, the number of fertile eggs, and the hatching rate of total eggs in red-legged partridges(Lopez-Antia et al. 2013). Furthermore, the fungicide thiram affected egg size and reduced clutch size, egg fertility, and brood size in red-legged partridges, with the highest concentrations of thiram producing mortality in 41.6% of birds(Lopez-Antia et al. 2015). Pyraclostrobin fungicide residues in crops have the potential to be harmful to the environment and human health (Huang et al. 2021). Fungicide Subacute mancozeb exposure in rats leads to elevated toxicity with impaired liver function, increased inflammation in tissue and increased apoptosis due to cellular damage in the liver, and decreased liver regeneration ability due to congestion and degeneration of blood vessels (Gök and Deveci 2022). Fungicide Mancozeb has been shown to cause adverse health effects in both humans and experimental animals. In humans, Mancozeb exposure was strongly associated with an increased incidence of thyroid disease in female spouses of pesticide applicators (Goldner et al. 2010). The toxicity of Mancozeb was also evidenced in several studies with different experimental models. In rats, Mancozeb caused general toxicity, thyroid hormone dysfunctions, oxidative stress, alterations in biochemical and hematological parameters, as well as neurotoxic and reprotoxic effects (Ahmed, Gamila and Kotb 2017, Bianchi et al. 2020, Pezzini et al. 2023). . In addition, occupational exposure to several fungicides was linked to a higher risk for neurodegenerative diseases (Parrón et al. 2011, Brouwer et al. 2017). A number of experimental studies have reported that acute and chronic exposure of birds to pesticides including organophosphate has adverse effect or even kill the birds immediately. The adverse effects of pesticides on the birds are behavioral, reproductive, and developmental that retarded their growth. Endocrine disruption, neurological changes, gastrointestinal problems, immunological response deficit, oxidative stress, and pathological lesions in different tissues are the other alteration observed among different birds (Brouwer et al. 2017, Suliman et al. 2020).

MATERIALS AND METHODS

The research was carried out in the Department of Zoology at The Islamia University of Bahawalpur, adhering strictly to the ethical guidelines and principles outlined by the departmental ethics committee for the use of animals in research. All necessary measures were taken to ensure the humane treatment and welfare of the experimental animals throughout the study.

Study Design

In current study apparently healthy sexually mature 28-day old adult Japanese quail (n= 30) approximately of equivalent weight as well as age were procured from the confidential market of Bahawalpur and they were reserved beneath parallel management state. Birds about 30 days old were brought into zoology laboratory of Zoology Department, the Islamia University of Bahawalpur, Pakistan. Quail acclimatized to ambient situation (room temperature (30±5°C), relative humidity (50 – 60%) as well as 12h light- dark cycle. Drinking water was accessible, basal diet was given to them in the morning as well as evening. Doses of fosetyl- aluminium 1ml/kg/day and 2ml/kg/day each bird were given to them through crop tube via opening mouth and insert tube with awareness into esophagus. Doses were injected according to body weight.

Experimental Procedure

After two week of acclimatization birds were arbitrarily separated into three evenly weighted corresponding groups. They named as G0, G1 and G2 respectively. Group G0 labeled as control group and received normal diet twice daily. Whereas Group G1 and Group G2 were considered as a experimental group. The different groups received oral administration of the different dosages of Fosetyl-Aluminium) by crop tubes daily for 30 days. On each day the feed and body weight were recorded, however on days 10, 20, and 30 of the experiment, average outcomes were obtained. Throughout the whole treatment period, we did not observe any disease in the birds, which were raised in a shed with a semi-controlled atmosphere.

Administration of Fosetyl-Aluminium

Fungicides (Fosetyl-Aluminium) concentrations were provided to different bird groups after acclimatization period. Group G0 labeled as control group and received distilled water with no fosetyl-aluminium for one week. Dissimilar doses of fosetyl - aluminium were given to the entire remaining groups. Dissimilar concentrations of doses were made according to the groups such as Group G1 (experimental group) received 1 g / 500ml water, whereas Group G2 (experimental group) received fosetyl-Al (2g/500ml of water).

Blood Sampling

Five birds from each group were randomly selected and euthanized by cutting the jugular vein on day 16, 32 and 48 of the experiment. Blood samples were collected from the severed vein into EDTA-coated and non-coated tubes for hematological and serum analysis, respectively. Following euthanasia, the birds were dissected, and their internal systems were exposed for examination. Postmortem observations, including the weights of the kidneys and liver, were recorded. These organs were then preserved in 10% buffered formalin for subsequent histopathological analysis.

Haemato-biochemical Parameters Analysis

During the pesticide exposure, birds from the control and experimental groups were chosen at random on days 10, 20 and 30 of the experiment. Five birds from each group were used to collect blood samples by cutting the jugular vein. Using a commercial haematology analyzer, hematological tests were performed on a blood sample to determine WBC, RBC counts, LYM, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean platelet volume (MPV), Plateletcrit (PCT), HGB, and mean corpuscular hemoglobin concentration (MCHC) in accordance with a previous study (Tchoupou-Tchoupou et al. 2022, Ahmed et al. 2023, Sindi et al. 2023, Ahmad et al. 2023). Blood samples with and without anticoagulant were gathered on the day of slaughtering. Approximately 5 ml of blood be collected in the fresh glass tube without anticoagulant and kept the tube in the sloping location for two to three hours for collection of serum. Collected serum was stored at -20°C for serum biochemistry. Serum samples were subjected to conclude the subsequent biochemical parameters included Urea (mg/dl), Creatinine (mg /DL), Bilirubin (mg /dl), ALT (SGPT) U/L and ALP U/L.

Statistical Analysis

Comparisons will be performed using the statistical software programs i.e., SPSS and Graph Pad Prism to check the effect of fungicide Fosetyl- aluminium against haematological and serum biochemistry in Japanese quail (*Coturnix japonica*). The data will be expressed as the mean ± SEM and comparisons between groups performed by using a t-test and one-way analysis of variance (ANOVA). All statistical analyses will perform using the statistical software 15. *P* values of *0.05* will regard as statistically significant.

RESULTS

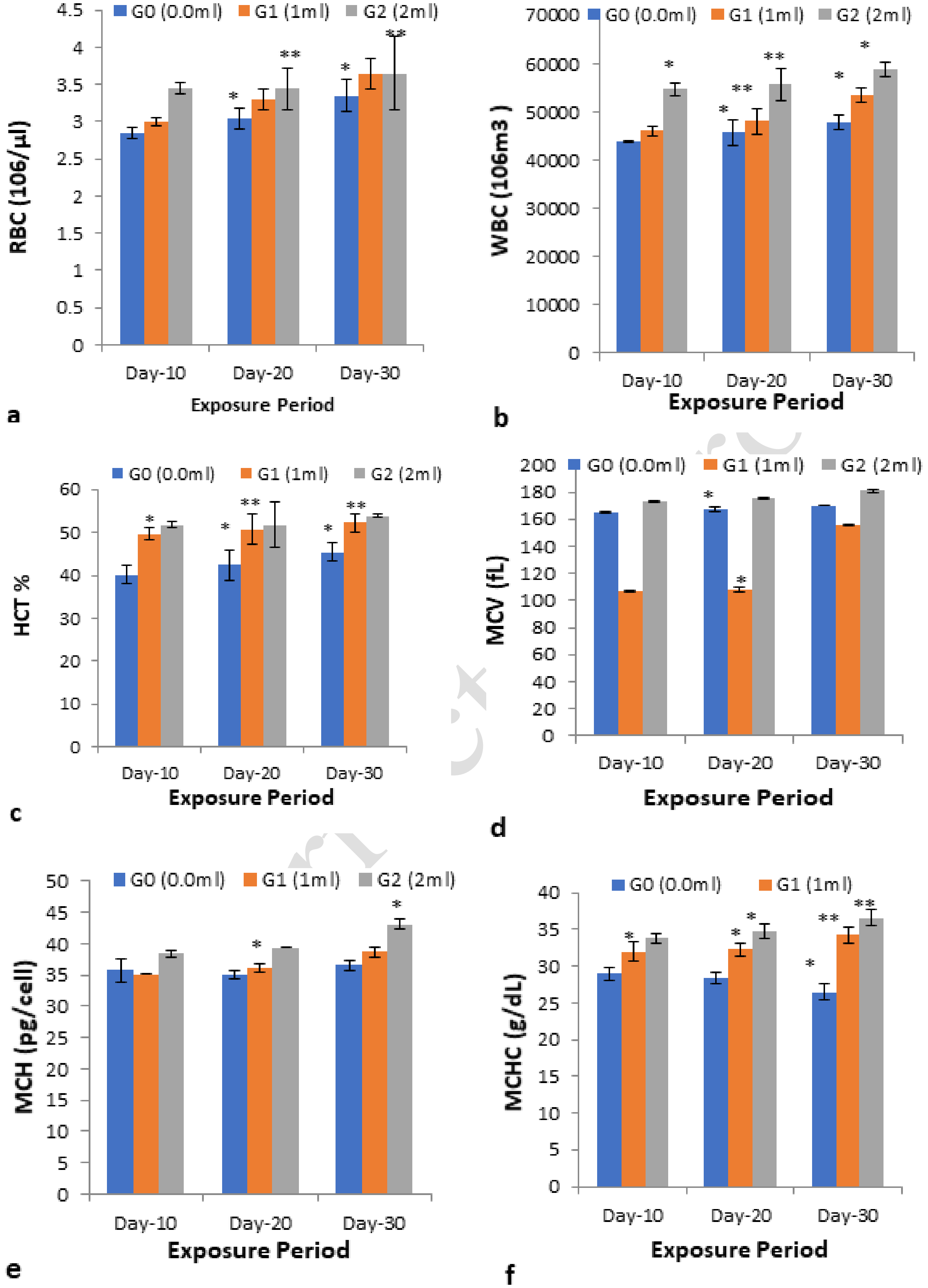
This study examined the toxic effects of fosetyl aluminium on the well-being of Japanese quail (*Coturnix japonica)* by assessing its hematological and serum biochemistry parameters. The findings of this current investigation are outlined in this study.

Hematological Responses

In a recent study, we examined the toxic effects of fosetyl aluminium on the hematological parameters of Japanese quails (*Coturnix japonica*). The results indicated notable alterations in hematological profiles due to exposure to this toxic substance. After 10 days of treatment, significant differences were observed in several hematological parameters between the control group and those exposed to fosetyl aluminium. Specifically, there were increases in white blood cell (WBC) counts, mean corpuscular hemoglobin concentration (MCHC), and platelet counts in the treated groups, particularly at higher doses. Conversely, parameters such as red blood cell (RBC) count, hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) did not show significant differences, suggesting that fosetyl aluminium selectively impacts certain physiological aspects of Japanese quails.

After 20 days of exposure to Fosetyl-Aluminum, notable differences were observed, including elevated levels of white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and lymphocyte percentage in the treated groups, especially at the high dose. In contrast, parameters such as red blood cell (RBC) count, hematocrit (HCT), and platelet count did not show any significant differences.

After 30 days of exposure to Fosetyl-Aluminum, notable differences were observed in various hematological


G0 (0.0ml)
G1 (1ml)
G2 (2ml)
RBC (106/µl)
Day-10
Day-20
Day-30
Exposure Period
a
WBC (106m3)
b
HCT %
c
MCV (fL)
d
MCH (pg/cell)
e
MCHC (g/dL)
f

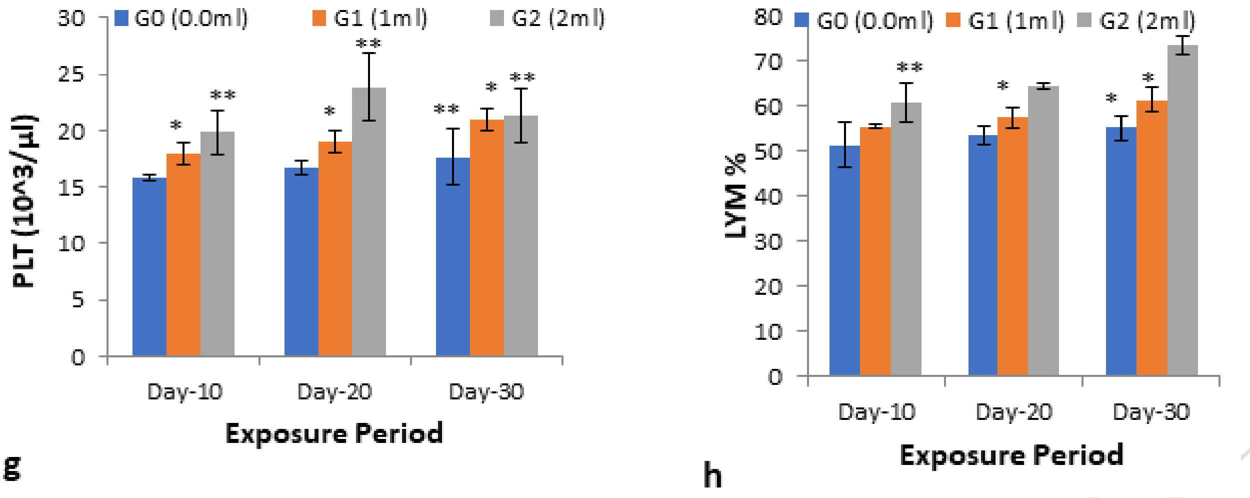


Fig. 1: Graphs representing hematological parameters with symbol (*) and (**) denotes significant deviation ($p<0.05$) between the control and experimental groups of*H. nobilis*, (a) RBCs count (106/µl), (b) WBC (106m3) (c) HCT (%), (d) MCV (fL), (e) MCH (pg/cell), (f) MCHC (g/dL), (g) PLT (10^3/µl), (h) LYM %.

parameters between the control and treated groups. A significant increase was recorded in parameters such as white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and lymphocyte percentage, particularly in the high-dose group. In contrast, parameters like red blood cell (RBC) count, hematocrit, and platelet count did not show significant differences, suggesting that Fosetyl-Aluminum has specific physiological effects on Japanese quail.

Biochemical Indices

Significant alterations in biochemical parameters were observed in Japanese quails exposed to Fosetyl Aluminum over a 10-day period. The treated groups, particularly those receiving higher doses, showed increased levels of urea, creatinine, alanine aminotransferase (ALT), and alkaline phosphatase (ALP). In contrast, bilirubin levels remained stable, indicating that Fosetyl Aluminum selectively impacts specific physiological functions in these birds. After 20 days of exposure, further notable changes were recorded, with elevated levels of urea, creatinine, bilirubin, ALT, and ALP particularly pronounced in the high-dose group. By the 30-day mark, the high-dose group continued to exhibit significant increases in urea, creatinine, bilirubin, ALT, and ALP compared to the control group. These findings suggest a progressive effect of Fosetyl Aluminum on the serum biochemistry of Japanese quails over time.

Table1. Biochemical Parameters in Japanese quail exposed to different doses of Fosetyl Aluminum in contrast with control.

DISCUSSION

Organophosphorous (OP) pesticides (insecticides, herbicides, and fungicides) are widely used in agriculture and for domestic pest management, causing a significant environmental risk (Ahmad, Khan and Khan 2012, Hussain, Khan and Mahmood 2013). Continuous exposure to these chemical substances not only adversely affects non-target organisms but also creates a hazardous situation for humans, primarily because of the residual presence of pesticides in crops and vegetables (Taylor et al. 2002). The lymphoid organs in developing avian species exhibit a high level of differentiation, which increases their susceptibility to toxic substances (Joshi et al. 2012, Ahmad et al. 2023). Fosetyl-Al is systemic fungicide widely utilized to safeguard various fruits and vegetables from plant pathogens (Gormez et al. 2022). It effectively controls foliar pests such as aphids, beetles, and caterpillars, as well as soil-dwelling pests like rootworms and cutworms (Tavaniello 2014). Fosetyl-aluminum resulted in a notable reduction in red blood cell count, hemoglobin concentration, and hematocrit levels in Japanese quail, suggesting potential health risks for avian species. In this study, we assessed the toxicity caused by the organophosphorus pesticide fosetyl-aluminum and its impact on the hematological and biochemical profiles of Japanese quails. The hematological changes observed from the analysis of blood variables after 10, 20, and 30 days of exposure have been documented. The hematological parameters measured included total erythrocyte count (RBC), hematocrit (HCT), hematological indices (MCHC, MCH, and MCV), as well as white blood cell count (WBC), lymphocytes (LYM), and platelet count (PLT). After a 10-day exposure to Fosetyl-Aluminum, there were notable increases in several hematological parameters, specifically white blood cell (WBC) count, mean corpuscular hemoglobin concentration (MCHC), and platelet count. However, no significant changes were observed in red blood cell (RBC) count, hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) during this period. By day 15, further significant increases were recorded in MCV, MCH, and MCHC, while the RBC count, HCT, and platelet count remained stable without any significant alterations. After 20 days of exposure, the trends continued with marked elevations in alanine aminotransferase (ALT), alkaline phosphatase (ALP), WBC, MCV, and MCHC. Again, there were no significant changes in RBC count, hematocrit, or platelet count during this time.

Parameters/days	Exposed groups		
	G0(0.0ml) Control	G1(1ml) Low	G2(2ml) High
Urea (mg/dL)			
10	9±0.14	11.3±0.56*	11.9±0.14**
20	8.71±0.61	11.5±0.56*	12.1±0.14**
30	8.8±0.56	12.3±0.28**	13.05±0.07**
Creatinine (mg/dL)			
10	0.1±0.01	0.4±0.02*	0.5±0.001**
20	0.15±0.01	0.4±0.02*	0.5±0.001**
30	0.15±0.07	0.55±0.07**	0.85±0.07**
Bilirubin (mg/dL)			
10	0.99±0.02	0.96±0.03	1.12±0.25*
20	1.05±0.05	0.96±0.03	1.12±0.25*
30	1.025±0.10	1.35±0.07*	1.85±0.07**
Alanine transaminase (IU/L)			
10	11±0	11.65±0.21*	13.8±0.14**
20	10.8±0.3	11.65±0.21*	13.8±0.14**
30	11.65±0.49	13.35±0.21**	16.5±0.42**
Alkaline phosphatase((IU/L)			
10	86.5±0.7	90.5±0.7*	92.5±0.7**
20	87.5±0.5	90.5±0.7*	92.5±0.7*
30	88±2.8	94.5±0.7*	99.5±2.1*

The notably reduced hematological values, including erythrocyte counts, hemoglobin levels, and hematocrit, may be linked to the induction of oxidative stress. This stress can lead to various abnormalities, such as myelotoxic effects and disruptions in heme production within the bone marrow of the exposed quail (Hussain et al. 2011, Hussain et al. 2014, Ghaffar et al. 2014). The increase in white blood cells (WBCs) and platelets may be due to enhanced antibody production (Malik and Maurya 2014). The rise in MCV and MCH values observed following pesticide treatment indicates that the reduction in RBC count may be due to either the destruction of red blood cells or a decrease in their synthesis in the bone marrow. Our findings align with those reported for Swiss albino mice and cockerels exposed to acetamiprid and other trichlorfon (Bagri et al. 2013, Hussain et al. 2021). Fosetyl-Aluminum toxicity profoundly affects serum biochemistry, causing elevations in liver enzymes like ALT and ALP, along with changes in Urea, Bilirubin, and Creatinine levels. Similarly, After 10 days of exposure to Fosetyl-Aluminum, there were notable increases in urea, creatinine, ALT, and ALP levels, while bilirubin levels did not show significant changes. By day 15, there were further significant increases in bilirubin. By day 20, the trends persisted, with significant elevations observed in urea, creatinine, bilirubin, ALT, and ALP. The enzymes ALT and AST are considered the most reliable biomarkers for assessing liver damage (Muhammad et al. 2012, Uzun and Kalender 2013). The elevated levels of these enzymes are a result of oxidative stress induced by pesticide treatment, leading to the destruction of hepatocyte cell membranes and the subsequent release of these enzymes into the bloodstream (Rahman et al. 2019). Transaminases, specifically AST and ALT, along with ALP, play a crucial role in regulating physiological processes by catalyzing transamination reactions that aid in the metabolism of xenobiotics and other macromolecules. Consequently, changes in their activity can directly indicate liver damage (Bacchetta et al. 2014). Our findings indicate that *Coturnix japonica* birds were vulnerable to all the parameters assessed after being exposed to fosetyl aluminium throughout the experiment. The research investigating the impact of fosetyl-aluminum on Japanese quails demonstrates notable toxic effects on their hemato-biochemical parameters. The results suggest that this substance, classified as an organophosphate pesticide, causes harmful changes in multiple organs of non-target species, such as birds. These alterations raise significant concerns regarding the potential risks to higher trophic levels, including humans, who occupy the top position in the food chain.

Conclusions

The study on the toxic effects of fosetyl aluminium on Japanese quails (*Coturnix japonica*) revealed significant alterations in hematological and biochemical parameters. The study highlighted the effects of these chemicals on different physiological markers, included increased white blood cell counts and biochemical markers like urea, creatinine, ALT, and ALP, indicating potential organ dysfunction. These results highlight the toxic effects of fosetyl aluminium on avian health and emphasize the need for caution in its agricultural use.

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Ethical Statement

This research was executed according to the guidelines of the ethical committee of the institution.

Availability of Data and Material

All the data is available and can be obtained on reasonable request from the corresponding author

Consent to Participate

All the authors equally participated in this research.

Consent for Publication

All the authors agreed and gave their consent for publication.

Competing Interest

Authors declare no conflict of interests.

Author Contribution

Sumaira Raziq: Conceptualization, Investigation; Formal Analysis, Writing -original draft. Adeeba Naseer: Data Curation, Investigation, Writing -original draft, Methodology. Visualization. Noman Waheed: Review and editing. Nousheen Ahmad: Review final draft. Nisha Ansari: Review final draft. Nimra Ather: Review final draft.

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