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TOXICOLOGICAL IMPACT OF CHLORPYRIFOS AND FOSETYL-ALUMINUM ON JAPANESE QUAIL: IMPLICATIONS FOR WILDLIFE CONSERVATION

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ABSTRACT

This research aims to analyze the impact of two pesticides, Chlorpyrifos and fosetyl-aluminum, on the hematological and biochemical profiles in the declining population of Japanese quail (*Coturnix japonica*) and situate its output within a wider field of ecological concerns and environmental education. The findings revealed profound and significant alterations of target mediators in a dose- and time-related manner. A total of 30 adult quails were divided into five groups: (G1) was treated as the control group, (G2) and (G3) were administered low doses, while (G4) and (G5) received high doses of Chlorpyrifos and fosetyl-aluminum, respectively. Each bird in the treated groups received an oral dose of 10 ml daily through a crop tube. After 10 days of exposure to both Chlorpyrifos and fosetyl-aluminum, significant changes were observed in urea, creatinine, ALT, ALP, WBC, and MCHC, with additional changes in bilirubin, MCV, and MCH appearing after 15 and 20 days. These physiological changes underscore the other effects of pesticides on general ecology since their use has ripple effects throughout trophic levels, affecting overall ecosystems. In addition to fundamental benefits from the cross-disciplinary analysis of the effects of toxicological damage on wildlife, the focus is on integrated, education-based approaches to advancing sustainable practices. The study also proposes that incorporating toxicological data into environmental education curricula will improve ecological understanding and the ability of communities to make wise decisions about the usage of pesticides.

1. INTRODUCTION

Chlorpyrifos (CP) and Fosetyl-Aluminum (Fs-Al) are commonly used pesticides in agriculture to control insects and fungi, respectively. However, long-term exposure to these pesticides is linked to negative health outcomes for both humans and animals, including cancer in humans, neurological abnormalities and developmental delays (Kumar et al., 2023). The study demonstrates the ecological concern linked to wildlife conservation.

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It contributes to a better awareness of the negative impacts of pesticide misuse, hence filling a gap in environmental education. The Japanese quail is used as a model organism in toxicological studies because of several reasons including its sensitivity to toxicity, declining population and short generation time. Earlier studies have proved that exposure to CP and Fs-Al can affect the hematology and biochemistry of these animals. By combining hematological and biochemical techniques, trial can be linked to a field based toxicant mechanism that impact population status. Optimal biomarkers such as platelets, hemoglobin, urea, creatinine and blood cell counts etc. can be used to assess physiological and chemical alterations in a variety of species following exposure to several kinds of harmful compounds, including pesticides and insecticides (Allasia 2023; Hussain et al., 2012). Any abnormal shift in level of these factors can be a sign of number illnesses including electrolyte imbalance, malnutrition, diabetes, liver or renal problems and many more. Consequently, serum biochemistry analysis stands as a pivotal tool in clinical medicine, aiding in the diagnosis, monitoring, and treatment of an array of diseases and health conditions (Konecka et al., 2023).

The Japanese quail reaches maturity in six weeks and often reaches maximum egg production by the time they are fifty days old (Mohammed & Ejiofor, 2015). Quail products are consumed as delicacies on festive occasions, and the domesticated Japanese quail, in contrast to its wild form, has lost all migratory behavior (Tavaniello, 2014).

Among the most widely used insecticides worldwide, organophosphorus pesticides (OPs) are widely available for commercial use in both residential and commercial settings. Worldwide, and especially in developing nations, the unregulated use of organophosphate (OP) pesticides increases the risk of pesticide poisoning. They effectively target foliar pests like aphids, beetles, and caterpillars, along with soil-dwelling pests such as rootworms and cutworms. Cp poses potential exposure risks through contaminated food and inhalation during processing, with systemic toxicity possible through skin absorption and inhalation of fumes during spraying. Although its residential use has ceased in the U.S. and the EU, residues of CP have been detected in produce, grains, and vegetables (Tavaniello, 2014). It works by inhibiting the activity of acetylcholinesterase, an enzyme that regulates the neurotransmitter acetylcholine in the nervous system. This leads to an accumulation of acetylcholine, which can overstimulate the nervous system and cause paralysis or death in insects. CP is toxic to both people and other animals. Numerous health problems such as nausea, headaches, dizziness, muscular and respiratory paralysis, can result from pesticide exposure. When taken in excess, might result in unconsciousness, seizures and even death. Recent years have seen controversy and regulatory initiatives due to their ecological concerns. In light of concerns over its effect on children's brain development, the U.S. Environmental protection Agency (EPA) has phased out its use on food crops, while various other agencies have restricted or even prohibited its usage (Wołejko et al., 2022).

The usage of pesticides has been shown to have adverse effects on biological diversity. It has been noted that pesticide use negatively affects biological diversity, and only 0.9% of these substances stay on their target insects. Pesticides have lethal and sub-lethal effects on non-target invertebrates affecting behavior, reproduction, enzymatic activity and DNA stability of earthworms, honeybees, predators and other parasitoids (Elhamalawy et al., 2024). The delicate balance between pests and their predators may be threatened by it.

This research attempts to explore the effects of sub-lethal dosages of two agrochemicals, CP and Fs-Al, on the health of Japanese quail by measuring changes in their hematology and biochemistry. The goal of this study is to comprehend the possible harm that these chemicals may cause to birds, which are useful as markers of health and may have wider implications for conservation of wildlife, ecological awareness and ecosystem balance.

2. MATERIALS AND METHODS

The study was conducted at the Department of Zoology, The Islamia University of Bahawalpur, Pakistan. A group of thirty adult Japanese quails (n=30) that apparently healthy and sexually mature, weighing between 90 and 100 grams and ranging in age from four to six weeks, were acquired from Ahmad-Puri Market in Bahawalpur. These quails were then placed in comfortable metal cages under uniform physical conditions: a photoperiod of approximately 12–14 hours of light, a room temperature of 27±5°C, and a relative humidity of 50–60%. All the birds had access to clean, fresh water and a commercially available standard diet (99 King Feeds) containing 20% protein, provided twice daily with an 8-hour interval.

The stock solutions were prepared from the chemicals Fosetyl-Al and CP, which were procured from M/S Bayers (Pvt.) Ltd. at Korangi Industrial Area, Karachi, and M/S Pak-China Chemicals Lahore, respectively. In this investigation, 2 mg of each chemical was added to 1000 mL of water to create high dosage solutions of CP and Fs-Al, which had a concentration of 0.002 mg/mL. One milligram of each compound was added to 1000 milliliters of water to create low dose solutions, which had a concentration of 0.001 mg/mL. Each bird received 10 mL of these solutions daily. Consequently, birds administered with the high dose received 0.02 mg of the chemical per day, while those given the low dose received 0.01 mg per day.

Throughout the acclimatization phase, all groups of experimental birds received water and food as per their usual routine, and a daily health check was conducted to monitor any signs of illness. Following the 7-day acclimatization period, the birds were divided into two main groups: the experimental group (n=24) and the control group (n=6), with the allocation being done randomly. The experimental group was further categorized into subgroups based on the type and concentration of the dose to be administered.

Each group consisted of 6 birds, making a total of 30 birds across all groups. Group 1 (G1) received fresh water and a standard feed twice a day throughout the experimental period. In Group 2 (G2), a low dose of Fs-Al was administered. Similarly, Group 3 (G3) birds were given a low dose of CP. Group 4 (G4) was treated with a high dose of Fs-Al, and Group 5 (G5) was given a high dose of CP. Each bird in the treated groups received an oral dose of 10 ml daily through a crop tube. The lethal dose (LD50) for the Japanese quail is about 15 mg/kg at 94.5% purity (Suliman et al., 2020).

Birds in the experimental group were randomly selected for euthanasia and sample collection by cutting the jugular vein on the 10th, 15th, and 20th days of the experiment. Blood was collected in EDTA vacutainers for hematological analysis and in non-EDTA vials for biochemical analysis. The blood samples were processed within 24 hours. All parameters were then analyzed statistically using one-way ANOVA with “IBM SPSS Statistics Version 27.”

3. RESULTS AND DISCUSSION

The treatment with CP significantly affected several hematological and biochemical parameters in Japanese quail, particularly at higher doses (1 mg). Notable changes were observed in urea ($P = 0.009$), creatinine ($P = 0.005$), bilirubin ($P = 0.048$), ALT ($P = 0.048$), ALP ($P = 0.002$), WBC ($P = 0.008$), MCV ($P = 0.001$), and granulocyte percentages ($P = 0.018$), indicating a dose-dependent response to CP exposure. Parameters like RBC count, HCT, MCH, MCHC, lymphocyte percentage, and platelet count showed no significant differences, suggesting selective impacts of CP on quail physiology (Table 1).

Pesticides pose significant threats to non-target organisms and ecosystems, with only a small fraction reaching intended pests. Adopting sustainable agricultural practices, such as precision agriculture and organic farming, can help mitigate the negative impacts of pesticides on agrobiodiversity and ecosystem health. Pesticides negatively impact crucial invertebrates like earthworms, honeybees, and beneficial predators, affecting their survival, behavior, and reproduction (Elhamalawy et al., 2024). Widespread use of pesticides contaminates soil, water, and food chains, leading to biodiversity loss and ecosystem disruption (Ali et al., 2021). Pesticides are widely used in agriculture for control of pests and the application of phytosanitary products, but they often contaminate water resources and soil through spray drift, runoff, and leaching (Barranger et al., 2014). Prolonged exposure to these chemical substances not only harms non-target organisms but also creates significant risks for humans, primarily due to the residual presence in vegetables and crops (Taylor et al., 2002).

Organophosphates (OP) are among the most extensively used chemicals in both agriculture and public health sectors (Ambali et al., 2010). They cause symptoms of poisoning, including tissue damage and neurotoxicity. In mammals and birds, the toxic effects of organophosphates arise from the inhibition of the cholinesterase enzyme is inhibited by OPs in mammals and birds which lead to its accumulation at neuromuscular junctions and nerve endings which affects central nervous system effects due to cholinergic overstimulation (Costa, 2006; Hamm et al., 1998; Richardson, 1995). Prolonged exposure to these insecticides has been shown to cause severe damage to vital organs.

Hemato-biochemical investigations play a crucial role in assessing the functional status of birds and animals exposed to toxic agents (Omitoyin, 2006). Since hemoglobin (Hb) and red blood cells (RBC) are linked to MCH and MCHC, So, variation in these factors have an immediate impact on MCH and MCHC which gives information about the size or condition of RBCs (Alwan et al., 2009). We noted a significant increase in the level of MCV, MCH, and MCH, while a marked decrease in RBC was counted. The increased rate of MCV and MCH demonstrated that the reduction in RBC count may be linked to either the destruction of RBCs or a reduced production in bone marrow. (Shakoori et al., 1992). Our findings are consistent with other studies on the effects of CP exposure to rats (Akhtar et al., 2009), fish (Ghayyur et al., 2019), broiler chicks (Ahmad et al., 2015) and pigeons ((Memon et al., 2024). Additionally, macrocytic anemia, characterized by larger-than-normal red blood cells, is indicated by a substantial increase in MCH and MCV levels (Nalina Thimmappa et al., 2019). A number of diseases, such as vitamin shortage and liver dysfunction, might be indicated by macrocytic anemia.

Shifts in WBCs count after being exposed to chemical stressors may be associated with a low immunity of organisms. Therefore, WBCs are a crucial component of blood in regulating response of Immune system to ailment (Ribeiro et al., 2006). Leukocytosis, an adaptive response to the chemical stress, is responsible for the rise in total WBC counts seen in Japanese quails following exposure to the chosen insecticides, CP and FS-Al (Ribeiro et al., 2006). Increased anti-body synthesis which helps animal exposed to chemical stressors, is also linked to WBC rise. After being exposed to CP similar findings were observed in house sparrows (Memon et al., 2024; Noreen et al., 2023; Yadav, 2017) and rats (Akhtar et al., 2009). In order to cope with tissue injuries caused by insecticides, other cockerels treated with OP previously displayed a higher number of WBCs (Hussain et al., 2012). Contrary to the present findings, significantly decreased WBC counts have also been documented in earlier investigations on exposure to CP (Shakoori et al., 1992).

By activating transamination events that aid in the metabolism of xenobiotics and other macromolecules, transaminases (AST and ALT) and ALP play important roles in controlling physiological processes. As a result, variations in their activity are clear signs of liver injury (Bacchetta et al., 2014; Fırat et al., 2011). In this study, a significant dose-dependent increase in liver enzyme levels such as ALP and ALT was recorded in Japanese quails after exposure to both CP and Fosetyl-Aluminium. Elevated activities of these enzymes signify liver damage, often due to increased permeability of hepatocyte membranes, which allows the enzymes to leak into the bloodstream. Additionally, higher enzyme concentrations are considered markers of recent organ damage rather than impaired organ function (Lumeij, 1997). Chlorpyrifos induced elevations of liver enzymes have also been observed in previous studies on birds such as broiler chicks(Ahmad et al., 2015) and indigenous chicken (Begum et al., 2015). Some researchers have also reported the liver toxicity induced by CP exposure in rats (Saoudi et al., 2021; Tanvir et al., 2016;Mansour & Mossa, 2010) .

Waste products of protein metabolism, urea and creatinine, are eliminated by the kidneys. A significant increase in plasma urea and creatinine levels in organisms treated with CP suggests kidney dysfunction and impaired glomerular filtration (Tanvir et al., 2016). Urea levels can also rise due to factors such as dehydration, the use of antidiuretic drugs, and dietary influences. However, high creatinine level specifically indicates the kidney damage (Garba et al., 2007). In this research, the exposure of specimens to selected organophosphorus pesticides, CP and Fs-Al, caused an increase in creatinine and blood urea especially when treated with higher concentrations, in comparison with control. This may be caused by defective glomerulus, increased disintegration of renal tissues, or reduced urinary clearance by the kidneys. The outcomes of this study line up with previous research, which also documented CP-induced elevations in blood urea and creatinine in rats (Saoudi et al., 2021; Tanvir et al., 2016) and mice (Ambali et al., 2007).

The integration of environmental literacy with ecotoxicological research is essential to address and cope up with global environmental challenges. The use of pesticides, particularly in the agriculture sector, poses notable health risks to communities and ecosystems. To diminish these risks, a multidimensional approach is needed, combining community-based learning, farm field schools, and strategic policies. The alignment of ecology and ecotoxicology is crucial to analyze and anticipate the impacts of contaminants on ecological system. This interdisciplinary approach also provides the foundation to assess the indirect effects of multiple stressors and stimuli on different biological processes.

According to the findings of our study, the hemato-biochemical metrics of Japanese quails were affected adversely by the toxic impacts of CP and Fs-Al; however, CP had more significant effects comparatively. These results determined that organophosphate pesticides induce transformations in different organs of non-targeted species like birds. These agrochemicals may even be lethal for human beings, who are at the top level of the food chain.

From a habitat management perspective, these findings emphasize the potential role of Japanese quail as a sentinel species for monitoring the ecological stress induced by the pesticides. The observed variations can serve as early warning indicators of environmental contamination, drawing attention to the need for systematic strategies on the use of pesticides. In addition, to cope up with the risks associated with irresponsible use of pesticides, certain conservation strategies, strict pesticide regulations and targeted educational initiatives are substantially imperative.

4. CONCLUSIONS

In conclusion, sub-lethal doses of Chlorpyrifos and Fosetyl-Aluminum adversely affect the health of non-target species, specifically Japanese quail, by altering serum biochemical and hematological parameters. These findings highlight more extensive risks of ecotoxicity to wildlife and the sustainability of ecosystems. The study uncovered the complex effects of these chemicals on several physiological markers. Notably, considerable rise in urea and creatinine levels indicate the compromised kidney function and dysregulated protein metabolism. Marginal changes in bilirubin and alkaline phosphatase (ALP) levels suggest minute disturbances in liver function, aligning with previous research. Moreover, there were notable alterations in red blood cells (RBCs) count, particularly in the groups exposed to high doses of chlorpyrifos. The fluctuations in white blood cells (WBCs) count and immunosuppressive effects were also detected. The Japanese quail serves as an ecological indicator species, demonstrating the impact of agrochemicals on wildlife and ecosystems. These outcomes emphasize the immediate need for public awareness regarding environmental toxicity and its ecological consequences. This paper urges policymakers, farmers, and the public to engage in responsible ecological management to neutralize biodiversity loss and preserve ecosystem integrity.

5. RECOMMENDATIONS

The current research study was an exploratory study conducted on the sub-lethal effects of CP and fosetyl aluminum on the hematological and biochemical parameters of Japanese quail. The pesticides and their broad-spectrum adverse effects pose threats not only to the quails but also other organisms such as chicken, rats, rabbits, and even humans. These pesticides can cause neural dysfunction and testicular impairment in addition to hematological and biochemical alterations. Moreover, they contribute to inducing oxidative stress and apoptosis. Given these interpretations, there is a pressing need for further in-depth research and immediate action to mitigate their detrimental effects. Reducing ecological imbalances requires promoting sustainable agriculture methods, such as the use of integrated pest management (IPM) techniques.

6. CONFLICT OF INTEREST

The author has declared that there is no conflict of interest regarding the publication of this article.

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Table 1. Comparison of the studied hematological and biochemical parameters between chlorpyrifos treated and untreated Japanese quail for a period of 10 days

Chlorpyrifos				
Parameter	**Control** **Mean ± SE**	**Low (0.01mg)** **Mean ± SE**	**High (0.02mg)** **Mean ± SE**	**P-Value**
Urea (mg/dL)	9±0.1	10.8±0.2	12±0.4	0.009*
Creatinine (mg/dL)	0.1±0	0.55±0.05	0.65±0.05	0.005**
Bilirubin (mg/dL)	0.995±0.015	1.15±0.05	1.4±0.1	0.048*
ALT (IU/L)	11±0	12.85±0.15	15.15±0.35	0.002**
ALP (IU/L)	86.5±0.5	89±1	103±1	0.002**
WBC ($10^6 m^3$)	45827.5±55.5	51870.5±2894.5	66187±689	0.008*
RBC (10^6/µl)	2.85±0.05	3.1±0.3	3.85±0.15	0.073
HCT %	41.45±1.55	50.2±4.4	51.65±2.05	0.163
MCV (fL)	166.85±0.65	175.95±0.55	180.65±0.55	0.001**
MCH (pg/cell)	35.725±1.325	36.35±1.45	40.05±0.15	0.134
MCHC (g/dL)	28.95±0.55	33.2±0.2	34.2±1.6	0.062
LYM %	51.5±3.5	60.5±0.5	64±2	0.067
GR %	30.5±1.5	37±2	47±2	0.018*
PLT (10^3/µl)	16.85±0.15	21.2±2.2	24.15±0.15	0.06

P <0.05 = Least Significant (*) P<0.01 = Significant (**)

The treatment with chlorpyrifos significantly affected several hematological and biochemical parameters in Japanese Quail, particularly at higher doses (1mg). Notable changes were observed in urea (P=0.009), creatinine (0.005), bilirubin (0.048), ALT (0.048), ALP (0.002), WBC (0.008), MCV (0.001), and granulocyte percentages (0.018), indicating a dose-dependent response to chlorpyrifos exposure. Parameters like RBC count, HCT, MCH, MCHC, lymphocyte percentage, and platelet count showed no significant differences, suggesting selective impacts of chlorpyrifos on quail physiology, (Table 1).

Table 2. Comparison of the studied hematological and biochemical parameters between chlorpyrifos treated and untreated Japanese quail for a period of 15 days.

Chlorpyrifos				
Parameter	**Control** **Mean** ± SE	**Low (0.01mg)** **Mean** ± SE	**High (0.02mg)** **Mean** ± SE	**P-Value**
Urea (mg/dL)	8.715±0.615	11.95±0.55	13.2±0.4	0.02*
Creatinine (mg/dL)	0.15±0.05	0.75±0.05	0.75±0.05	0.005**
Bilirubin (mg/dL)	1.05±0.05	1.45±0.05	1.8±0.1	0.011*
ALT (IU/L)	10.8±0.3	13.85±0.65	16.5±0	0.005**
ALP (IU/L)	87.5±0.5	93±2	107±2	0.008**
WBC (10^6m^3)	45815±939	52990±2442	67987.5±444.5	0.004**
RBC (10^6/µl)	3.25±0.45	2.95±0.05	3.6±0.2	0.393
HCT %	43.65±1.55	50.65±4.55	51.6±3	0.312
MCV (fL)	169.6±0.8	176.6±0.8	183±0	0.002**
MCH (pg/cell)	35±0	38.65±0.95	41.9±0.5	0.01*
MCHC (g/dL)	29.3±1.3	34.15±0.05	36.55±1.85	0.063
LYM %	51.5±0.5	65.5±2.5	71±2	0.011*
GR %	30.5±0.5	40±3	50.5±2.5	0.019*
PLT (10^3/µl)	17.65±1.65	22.6±1.9	24.2±1.3	0.129

P <0.05 = Least Significant (*) P<0.01 = Significant (**)

After 15 days of exposure, significant differences in several hematological and biochemical parameters were observed between the control and CP-treated groups. Notable changes included increased levels of urea (0.02), creatinine (0.005), bilirubin (0.011), ALT (0.005), ALP (0.008), WBC (0.004), MCV (0.002), MCH (0.01), lymphocyte percentage (0.011), and granulocyte percentage (0.019) in the treated groups, particularly at the high dose. Parameters such as RBC count, HCT, MCHC, and platelet count showed no significant differences, indicating selective impacts of CP on specific physiological aspects of Japanese quail (Table 2).

Table 3. Comparison of the studied hematological and biochemical parameters between chlorpyrifos treated and untreated Japanese quail for a period of 20 days.

Chlorpyrifos				
Parameter	**Control** **Mean ± SE**	**Low (0.01 mg)** **Mean ± SE**	**High (0.02 mg)** **Mean ± SE**	**P-Value**
Urea (mg/dL)	8.8±0.4	13.1±0.6	15.7±1.2	0.02*
Creatinine (mg/dL)	0.15±0.05	1±0.1	1±0.1	0.009**
Bilirubin (mg/dL)	1.025±0.075	1.7±0.1	2.05±0.05	0.006**
ALT (IU/L)	11.65±0.35	15.1±0.6	17.75±0.35	0.006**
ALP (IU/L)	88±2	97.5±0.5	109±1	0.004**
WBC (10^6m^3)	47780±985	55209.5±2444.5	70148.5±394.5	0.004**
RBC ($10^6/\mu l$)	3.05±0.05	3.15±0.05	3.5±0.4	0.461
HCT %	44.3±0.5	51.55±4.25	51.5±3.5	0.32
MCV (fL)	172.1±0.5	176.85±0.65	185.5±1	0.002**
MCH (pg/cell)	36.5±2.5	39.4±0.8	45.2±0.6	0.062
MCHC (g/dL)	27.5±0.5	35±0.7	38.75±1.25	0.006**
LYM %	53±3	66.5±4.5	75±1	0.036*
GR %	29.5±1.5	39±5	53±2	0.032*
PLT (10^3/µl)	18.6±1.4	25.1±1.6	25.6±2	0.102

P <0.05 = Least Significant (*) P<0.01 = Significant (**)

After 20 days of exposure to CP, significant differences in several hematological and biochemical parameters were observed between treated and control groups. The treated groups, particularly those receiving the high dose, exhibited notable increases in urea (0.020), creatinine (0.009), bilirubin (0.006), ALT (0.006), ALP (0.004), WBC (0.004), MCV (0.002), MCHC (0.006), lymphocyte percentage (0.036), and granulocyte percentage (0.032). In contrast, parameters such as RBC count, HCT, MCH, and platelet count showed no significant differences, highlighting the selective effects of CP on specific physiological aspects of Japanese quail (Table 3).

Table 4. Comparison of the studied hematological and biochemical parameters between fosetyl-aluminum treated and untreated Japanese quails for 10 days.

Fosetyl-Aluminum				
Parameter	**Control** **Mean ± SE**	**Low (0.01mg)** **Mean ± SE**	**High (0.02mg)** **Mean ± SE**	**P-Value**
Urea (mg/dL)	9±0.14142	11.3±0.56569	11.9±0.14142	0.007**
Creatinine (mg/dL)	0.1±0	0.4±0	0.5±0	0.013**
Bilirubin (mg/dL)	0.995±0.02121	0.965±0.03536	1.12±0.25456	0.599
ALT (IU/L)	11±0	11.65±0.2121	13.8±0.1414	0.001**
ALP (IU/L)	86.5±0.707	90.5±0.707	92.5±0.707	0.008**
WBC ($10^6 m^3$)	45827.5±78.489	47999±2654.479	54664±1428.356	0.03*
RBC (10^6/µl)	2.85±0.0707	3±0.1414	3.45±0.2121	0.059
HCT %	41.45±2.192	50.7±3.5355	51.75±2.0506	0.054
MCV (fL)	166.85±0.9192	109.75±91.5703	178.4±0.4243	0.474
MCH (pg/cell)	35.725±1.87383	35.15±0.6364	38.4±0.70711	0.139
MCHC (g/dL)	28.95±0.7778	31.95±0.7778	34.8±1.1314	0.018**
LYM %	51.5±4.95	55.5±2.121	61±2.828	0.155
GR %	30.5±2.121	33.5±0.707	40±2.828	0.042*
PLT (10^3/µl)	16.85±0.2121	19.95±0.6364	23.85±2.4749	0.04*

P <0.05 = Least Significant (*) P<0.01 = Significant (**)

Significant alterations were observed in several hematological and biochemical parameters between control and treated groups of Japanese quails after 10 days of exposure to Fs-Al. Notable changes included increased levels of urea (0.007), creatinine (0.013), ALT (0.001), ALP (0.008), WBC (0.03), MCHC (0.018), granulocyte percentage (0.042), and platelet count (0.04) in the treated groups, particularly at the high dose. However, parameters such as bilirubin, RBC count, HCT, MCV, MCH, and lymphocyte percentage did not show significant differences, indicating that Fs-Al selectively affects specific physiological aspects of Japanese quails (Table 4).

Table 5. Comparison of the studied hematological and biochemical parameters between fosetyl-aluminum treated and untreated Japanese quails for 15 days.

Fosetyl-Aluminum				
Parameter	**Control** **Mean ± SE**	**Low (0.01mg)** **Mean ± SE**	**High (0.02mg)** **Mean ± SE**	**P-Value**
Urea (mg/dL)	8.715±0.615	11.3±0.56569	11.9±0.14142	0.012**
Creatinine (mg/dL)	0.15±0.05	0.4±0	0.5±0	0.013**
Bilirubin (mg/dL)	1.05±0.05	0.965±0.03536	1.12±0.25456	0.041*
ALT (IU/L)	10.8±0.3	11.65±0.2121	13.8±0.1414	0.001**
ALP (IU/L)	87.5±0.5	90.5±0.707	92.5±0.707	0.011*
WBC ($10^6 m^3$)	45815±939	47999±2654.479	54664±1428.356	0.012**
RBC (10^6/µl)	3.25±0.45	3±0.1414	3.45±0.2121	0.487
HCT %	43.65±1.55	50.7±3.5355	51.75±2.0506	0.308
MCV (fL)	169.6±0.8	109.75±91.5703	178.4±0.4243	0.01**
MCH (pg/cell)	35±0	35.15±0.6364	38.4±0.70711	0.01**
MCHC (g/dL)	29.3±1.3	31.95±0.7778	34.8±1.1314	0.025*
LYM %	51.5±0.5	55.5±2.121	61±2.828	0.006**
GR %	30.5±0.5	33.5±0.707	40±2.828	0.024*
PLT (10^3/µl)	17.65±1.65	19.95±0.6364	23.85±2.4749	0.497

P <0.05 = Least Significant (*) P<0.01 = Significant (**)

After 15 days of exposure to Fs-Al, significant differences were noted in several parameters among the treated groups, especially at the high dose. Increased levels were observed for urea (0.012), creatinine (0.013), bilirubin (0.041), ALT (0.001), ALP (0.011), WBC (0.012), MCV (0.01), MCH (0.01), MCHC (0.025), lymphocyte percentage (0.006), and granulocyte percentage (0.024). In contrast, parameters such as RBC count, HCT, and platelet count showed no significant differences (Table 5).

Table 6. Comparison of the studied hematological and biochemical parameters between Fosetyl-Aluminum treated and untreated Japanese quails for 20 days.

Fosetyl-Aluminum				
Parameter	**Control** **Mean ± SE**	**Low (0.01mg)** **Mean ± SE**	**High (0.02mg)** **Mean ± SE**	**P-Value**
Urea (mg/dL)	8.8±0.56569	12.3±0.28284	13.05±0.07071	0.003**
Creatinine (mg/dL)	0.15±0.0707	0.55±0.0707	0.85±0.0707	0.005**
Bilirubin (mg/dL)	1.025±0.10607	1.35±0.07071	1.85±0.07071	0.005**
ALT (IU/L)	11.65±0.495	13.35±0.2121	16.5±0.4243	0.003**
ALP (IU/L)	88±2.828	94.5±0.707	99.5±2.121	0.027*
WBC ($10^6 m^3$)	47780±1393	53518±3211.679	58709.5±1492.702	0.036*
RBC (10^6/µl)	3.05±0.0707	3.6±0.2828	3.55±0.495	0.324
HCT %	44.3±0.7071	50.25±5.3033	49.95±0.495	0.245
MCV (fL)	172.1±0.7071	175.6±0.8485	180.8±0.8485	0.004**
MCH (pg/cell)	36.5±3.53553	38.65±0.07071	43.05±0.77782	0.108
MCHC (g/dL)	27.5±0.7071	34.25±1.0607	36.6±1.1314	0.006**
LYM %	53±4.243	61.5±0.707	73.5±2.121	0.012**
GR %	29.5±2.121	35±1.414	45±2.828	0.013**
PLT (10^3/µl)	18.6±1.9799	20.95±5.0205	21.3±2.4042	0.718

P <0.05 = Least Significant (*) P<0.01 = Significant (**)

After 20 days of exposure to Fosetyl-Aluminum, notable differences were observed in various hematological and biochemical parameters between the control and treated groups. Significant increases were recorded in urea (p=0.003), creatinine (p=0.005), bilirubin (p=0.005), ALT (p=0.003), ALP (p=0.027), WBC (p=0.036), MCV (p=0.004), MCHC (p=0.006), lymphocyte percentage (p=0.012), and granulocyte percentage (p=0.013), particularly in the high-dose group. In contrast, RBC count, hematocrit, and platelet count showed no significant differences, highlighting the specific physiological effects of Fs-Al on Japanese quail (Table 6).