



Effect of Microplastic Ingestion on Digestive Enzymes, Hormones, Hematology Serum Biochemistry of *Gallus gallus domesticus*



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Abstract

THE CURRENT study aimed to determine how microplastics affect domestic chickens' growth rate, body temperature, digestive enzymes, hormone levels, hematology, and serum biochemistry. In the growth and body temperature record, bird body weight gain was minimum body temperature was maximum in group three (G3) as compared to others. The hematological parameters i.e. total white blood cell count (TWBC) count and mean corpuscular volume (MCV) were highest in G3 while, total red blood cell (TRBC) count and packed cell volume (PCV) were highest in G2. The creatinine kinase (CK-NAC), aspartate transaminase (ASAT), and urea showed maximum value in G3 while cholesterol, total protein, albumin, alanine aminotransferase (ALAT), and uric acid level was highest in G2. A significant increase in the level of amylase and total protease enzymes and all hormones i.e. luteinizing hormone (LH), thyroid stimulating hormone (TSH), follicle-stimulating hormone (FSH), triiodothyronine (T3), tetraiodothyronine (T4) was observed in G3 while the production of lipase enzyme remains unaffected. Overall, the experimental groups showed higher hormonal levels and enzymatic production while decrease in body weight gain which might suggest that food contaminants eventually lower chickens' net energy intake from food they consume and their energy reserves.

Keywords: Microplastic, Chicken, Body temperature, Growth performance, Hormonal levels, Digestive enzyme, Serum biochemistry, Hematology.

Introduction

Since the 1950s, there's been a noticeable rise in the commercial enterprise and use of plastic goods due to growing industrialization and modernization [1, 2]. Plastic particles with diameters less than 5 mm are known as microplastics (MPs) and smaller than 1µm as nanoplastics (NPs). These particles can get into the food chain through ingestion or inhalation and can be found in a variety of forms, structure, and quality in terrestrial, marine, and atmospheric environments [3]. Numerous wild creatures can consume them since they are bioavailable [4, 5, 6] and can trophically move into food chains, posing serious risks to ecosystems and biodiversity [7, 8, 9]. As a result, amount of plastic garbage along with plastic trash in the environs has continued to rise, causing significant environmental contamination that

eventually has an effect on animal health either directly or indirectly [10-12].

Birds being endotherms are widely dispensed in various habitats worldwide have a high metabolic rate [13] and antioxidant capacity [14] extended lifespan and an effective digestive system [15]. Since they are thought of as extremely sensitive to outside factors, they might be used to track changes in the environment and evaluate the detrimental impacts of pollution [16-18]. Several species of birds from freshwater, terrestrial, and marine environments have been shown to have MPs in their feathers, feces, and gastrointestinal tracts [11, 19, 20]. There is evidence that consuming microplastics may cause birds to experience a range of adverse impacts, such as alterations in body weight [21], body temperature [22], digestive enzymes activity [23], hormonal activity [24], hematology, and serum biochemistry

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[25]. However, the variety and quantity of microplastics consumed, as well as the species of bird, may have different consequences.

Gallus gallus domesticus (Galliformes, Phasianidae) is a highly popular domestic animal worldwide [26] have been regarded as effective experimental model organisms in investigations on ecological toxicology [27, 28, 29] due to its sensitivity, quick reaction to environmental toxins, and expression of outward signs as a marker of toxic exposure, including as body weight, behavioral response, egg production, and shell thickness.

The current work set out to find if the exposure of chicks (*Gallus gallus domesticus*) to microplastics could provide a health concern. It is hypothesized that, even at low concentrations and for a short period of exposure, microplastics can induce changes in birds' weight gain, body temperature, digestive enzymes activity, hormonal activity, hematology, and serum biochemistry, as these parameters are commonly used as systemic toxicity biomarkers.

Material and Methods

Experimental Design

The experimentation was carried out in accord with the institutional policies on the attention and euthanasia of animals. A total of thirty-two chicks weighing an average of four hundred grams were bought from the neighborhood market and placed in an animal house facility at the Botanical Garden BZU, Multan, for sixteen weeks. All the birds were closely examined and immunized against common poultry diseases prior to the outset of the trial. The birds were fed on a regular basis and given access to food and water *ad libitum*. The experimental trial started after the birds had been acclimated for two weeks.

The birds were divided into four groups, each containing eight chicks. One group was kept as a control, and three were experimental groups given microplastics or microbeads mixed with flour. A dough was prepared from the above mixture, and semi-cooked bread was prepared for bird consumption in the form of small granules. Microplastics were given at a rate of 20%, 30%, and 40% of their daily diet. The microplastics were extracted from cosmetics and daily personal care products containing synthetic chemicals in their ingredient formulations (glycerylmonostearate, ethylene glycol istearate, cetearly alcohol, propyl paraben, methyl paraben, propylene glycol, propyl paraben, polysorbate 20, polyquaternium, methyl paraben, methyl ester, ethyl esters, propyl ester, propylene, polysorbate, linolene, and carbomer).

Measurement of temperature and body weight

Digital thermometer and weighing scale were used for measurement of body temperature and body weight on weekly basis.

Blood Collection and Gut extraction

At completion of animal trial, blood was collected from wing vein and stored into EDTA tubes for hematological and serological analyses while chicks were autopsied, and gut was extracted for digestive enzymes analysis. The whole gut was taken out onto an ice tray, cleaned of any debris or lipids with a child Tris-HCl buffer, wrapped into the aluminum foil and frozen to allow for the safe removal of gastric enzymes.

Enzyme Extraction and Quantitative evaluation

The previously stored samples were homogenized in Tris-HCl buffer in a homogenizer before being subjected to enzyme extraction and quantitative evaluation. The homogenate was centrifuged in an ultra-centrifuge @ 15,000 rpm for 30 minutes at 4°C. After being extracted, the supernatants were frozen until analysis.

The approach of Smith and Roe [30], which was later improved by Howard and Yudkin [31], was used to determine the activity of amylase. The definition of the activity was 10 mg of starch hydrolyzed in 30 minutes at 37 °C by 1 g of enzyme. A starch solution was made, enzyme homogenate was added, after which a waterbath was used to incubate the mixture. The absorbance at 540 nm wavelength was measured using a Spectrophotometer U-2900. Teitz and Fiereck method were used to study lipase activity in gut samples. Samples were titrated with standard 2N NaOH solution until the color turned pink, after being incubated for 30 minutes at 37°C with olive oil substrate and phosphate buffer. For protease analysis of gut homogenate 0.65% casein was used as substrate [32], followed by incubation with enzyme homogenate and Tri-chloro acetic acid. The absorbance of the filtrate was recorded at 660nm, and the absorbance values were compared to the standard curve to determine protease activity.

Blood Hormones

The levels of FSH, LH, TSH, T3, and T4 were determined by using the enzyme-linked immunosorbent assay (ELISA) and according to the manufacturer's guidelines [33].

Serum Biochemical and Hematological Analysis

Serum concentration of cholesterol, creatine kinase, total protein, albumin, aspartate transaminase, alanine transaminase, urea and uric acid were determined by using auto analyzer Micro-lab 200 made by Diasys. A micro-hematocrit capillary tube spun at 10,000 rpm was utilized to find out the packed cell volume, and the height of blood plasma was used to compute the hematocrit. The total red

blood cells and white blood cells were determined manually through Neubauer Ruling Hemacytometer. While mean corpuscular volume is calculated from hematocrit and red blood cells count.

Statistical analysis

Analysis of variance (ANOVA) was used to compare the data at a significance level of 0.01. Every reading was reported as Mean \pm SD. For all statistical studies, SPSS (Statistical Package for the Social Sciences) version 17.0 was utilized.

Results

In this study, the effect of microplastic on body weight, hematology, serology, hormonal and digestive enzymes activities of *Gallus gallus domesticus* was assessed. Body weight gain was observed during 16 weeks of trial after feed ingestion containing microplastic. Body weight gain ranged from 25 \pm 20.41 to 110 \pm 33.67, 30 \pm 5 to 123.33 \pm 5.78, 18.75 \pm 12.5 to 91.25 \pm 18.23gm in G1, G2 and G3 respectively. A significant difference in growth pattern was observed among all groups with minimum weight gain 50.16g in G3 as compared to other groups. The average weekly body weight gain has been shown in table 1.

The average body temperature also showed variation in all experimental groups ranged from 103.85 \pm 0.35 to 107.93 \pm 0.83, 104.73 \pm 0.39 to 108.35 \pm 0.13, and 104.1 \pm 0.22 to 108.95 \pm 0.48 in G1, G2 and G3 respectively. Because of these variations, it is assumed that different level of microplastic in bird feed had a significant influence on body temperature as maximum average temperature was recorded in G3 (108.95 $^{\circ}$ F) with high percentage of microplastics in their feed. The average weekly temperature variation has been shown in Table 2.

The activity of various digestive enzymes i.e. amylase, protease and lipases were significantly affected by the ingestion of microplastic. Average value of amylase activity was 1.36 \pm 0.18, 1.66 \pm 0.5, 2.21 \pm 0.51, and 2.52 \pm 0.46 U/mL.min-1 in control, G1, G2, and G3 respectively. Maximum amylase activity was observed in G3, while minimum in control group. Similarly, the activity of amylase varied as 1.85 \pm 0.13, 1.91 \pm 0.49, 2.14 \pm 0.58, and 2.32 \pm 0.58 U/mL.min-1 in control, G1, G2, and G3 respectively with maximum in G3. Lipase activity was found 0.61 \pm 0.07 in control group, while 0.606 \pm 0.24, 0.605 \pm 0.25, and 0.606 \pm 0.23 U/mL.min-1 in G1, G2, and G3 respectively. Activity of amylase and protease increased significantly with increase in ingestion of microplastic, while lipase remained consistent in all study groups. The activities of different digestive enzymes in the experimental and control groups of *Gallus gallus* (Fig. 1).

Hormonal activities showed variation in different studied groups. The average value of LH was

calculated found 6 \pm 0.26 in control, while 7.3 \pm 0.15, 8.5 \pm 0.2, and 9 \pm 0.264 ng/ml in the G1, G2 and G3 respectively with maximum in G3. FSH was 100 \pm 2, 102 \pm 2, 105 \pm 2, and 107 \pm 2 mIU/mL in the control, G1, G2 and G3 respectively, while TSH was 0.6 \pm 0.2, 0.7 \pm 0.2, 0.8 \pm 0.2, and 0.9 \pm 0.2 ng/dL in the control, G1, G2 and G3. The average value of T3 increased with increase in microplastic ingestion as 1.9 \pm 0.2 nmol/L in G3, similarly in T4 with maximum value of 1.76 \pm 0.02 nmol/L in G3. All hormones (LH, TSH, FSH, T3, and T4) have the highest mean values in G3 with maximum microplastic ingestion compared to the all other studied groups (Fig. 2).

TRBC, TWBC, MCV, and PVC also showed variation in study groups as the average value of TRBC, TWBC, MCV, and PVC was 3.42 \pm 0.24, 0.075 \pm 0.004, 74.85 \pm 8.18, and 24.96 \pm 0.78 respectively in control group, while 4.1 \pm 0.84, 0.10 \pm 0.012, 59.15 \pm 10.2, and 24 \pm 3.38 respectively in G1. For G2 the average values were found 4.172 \pm 0.887, 0.0996 \pm 0.021, 63.4 \pm 12.2, and 26.64 \pm 2.78 and 3.494 \pm 0.356, 0.10 \pm 0.0063, 69.75 \pm 6.38, and 23.6 \pm 1.36 respectively in G3. A variation in hematological indices was observed under different groups with maximum TRBC, and PCV in G2, TWBC and MCV in G3 with significant differences (Fig. 3).

Cholesterol, Total Protein, Albumin, ALAT/GPT and Uric acid was found maximum in G2 with average value of 124.4 \pm 15.8 mg/dl, 4.3 \pm 1.45 g/dl, 2.84 \pm 0.872 g/l, 8.8 \pm 1.77 U/L, and 904 \pm 162 μ mol/L respectively, while CK-NAC, ASAT/GOT and Urea was found maximum in G3 with average value of 1371 \pm 541 U/L, 295.6 \pm 14.6 U/L, and 3.71 \pm 0.275 mmol/L respectively. A significant difference was observed in different groups such as CK-NAC, ASAT/GOT and Urea value found increases with microplastic ingestion (Fig. 4).

Discussion

In the present case, mixture of microplastics used in cosmetic products were mixed in dough and given to animal in the form of semi cooked chapatti. During the experimental period of 16 weeks, the processed feed depicted an adverse impact on both body temperature and weight. This unequivocally shows that a bird's body weight and temperature are negatively correlated with its ingestion of microplastics. It was found that the chicks in groups 3 (given a maximum quantity of microbeads of 40%) had a minimum weight gain as compared to group 1 and 2 (provided a minimum and medium quantity of microplastics/microbeads, respectively, of 20% and 30%). Apart from group 1, there was a noticeable difference between the experimental and control groups' body weights. Overall, insufficient food digestion and absorption efficiency may be the cause of such noticeable weight variations. A similar pattern was observed by Ryan [21] who found that

chickens exposed to microplastics in feed had reduced body weight gain and poorer feed conversion efficiency as compared to those were not exposed to such kind of impurities in feed. It is important to remember that eating is hampered by consuming large amounts of microplastic since meals become shorter. According to Connors and Smith [34], this may lessen the body's capacity to accumulate fat reserves needed for molting, migrating, and reproducing.

The maximum body temperature of the experimental and control groups was recorded in G3 followed by G2. High temperature was observed at high dose level while normal at low dose level i.e. G1. If organisms ingest a toxicant, such as a chemical or heavy metal, their body temperature may increase due to the activation of their immune system and inflammation known as an immune response or an inflammatory response [22]. The immune system responds to the presence of a foreign substance in the body by producing inflammatory molecules, such as cytokines and prostaglandins, which help destroy the toxicant and facilitate body protection. However, these molecules can also cause the body temperature to rise, as they increase blood flow to the affected area and stimulate the production of heat [35].

Although the precise processes are unclear, neuroendocrine systems control the concentrations of digestive enzymes in the body of birds [36]. Typically, dietary substrates are recognized chemically, causing the epithelial cells lining the digestive tract to produce and release the enzymes required for digestion [37]. This regulation tightly coordinates substrate availability and enzyme concentrations to maximize Gallus's net energy from a meal [38]. If the hydrocarbons that compose the plastic polymer or molecular leachates of microplastics mimic the substrates at the molecular level, feeding microplastics may cause an up-regulation of enzymes [39]. When substances obstruct substrate-detecting systems or cause physical or chemical damage to the secretory epithelial cells, the quantities of digestive enzymes decrease [40].

The increased amylase activity was observed in all experimental groups with the highest value in G3. Amylase appeared to be sensitive to microplastics. It was discovered that amylase activity was not affected by microplastic mixes [41]. Though an increase in amylase level observed may be the result of a mechanism involving microplastic leachates, as all polymers leach chemicals. However, endogenous amylase upregulation may be triggered by microplastic leachates [42]. Potentially, carbohydrate rich food items such as flatbread may also be a good reason for amylase increment. Changes in food content and quality can be responded to by the gastrointestinal system through the modification of digestive enzyme activity [43]. The birds exposed to

microplastics while consuming a different kind of meal (i.e. chappati a form of carbohydrate) would display difference in amylase activities as seen in present case.

All treatment groups had considerably increased protease activity, with G3 having the highest value. Because the protease detection assay utilized in this investigation was non-specific, our measure encompasses both intracellular proteases that break down cellular components and digestive proteases which act on ingested proteins. Changes in intracellular protease concentration are thought to be reflected in the changed protease in this treatment. The amount of microplastics in the test diet wore down the digestive glands' epithelium as recorded [44, 45]. In this sense, tissue injury from the microplastic therapy might have set off intracellular protease activity. The current study's protease results contradict the lower levels of trypsin and pepsin noted by Wang *et al.* [23]. Nevertheless, given that different proteases react to microplastics in different ways [46], it's plausible that the decreases in trypsin and pepsin in our investigation were obscured by increase in other proteases.

Microplastic treatments did not significantly alter lipase activity in any experimental group. This corroborates the observations of [47], who outlined that lipase was unaffected by exposure to microplastics. Because the surfaces of microplastics absorb the enzymes and their substrates, the size and corresponding surface area of microplastics can block lipases [48]. Potentially the Tween20/Polysorbate, used frequently as emulsifying agent in detergents and cosmetics, is the reason for this ineffectiveness. Lipases and lipids would be repelled by the micelle of hydrophilic molecules that Tween20 forms that faces outward [49]. Being present in large quantities in the experimental feed, it acted as a surfactant and prevented lipases and lipids from adsorption hence an overall lipase activity remained unaffected.

Current study showed a significant increase in the level of LH, TSH, FSH, T3 and T4 in chicks exposed to microplastics in their diet. The highest mean values of all hormones were observed in G3 exposed to 40% microplastic of their diet. These findings align with past studies conducted in various pesticide-related work environments. In this case, it was found that pesticide exposure positively linked with levels of FSH and LH [24]. LH and FSH are released when the hypothalamus and pituitary exercise feedback control over testosterone levels. Furthermore, the pesticides boost the biosynthesis and synthesis of hypothalamic gonadotropin-releasing hormone, which in turn boosts the pituitary's release of LH and FSH [50].

Many studies have connected the ingestion of microplastics to toxicity to bird reproductive [51].

When male Japanese quail chicks were seen eating plastic, for example, they displayed a slightly delayed level of maturity and a higher frequency of epididymal intraepithelial cysts [51]. In another experiment, an adult male Rose-ringed parakeet (*Psittacula krameri*) treated to methyl parathion displayed decreased testicular function, which could be linked to altered levels of testosterone and LH in the blood [52]. The high hormonal level in the *Gallus gallus domesticus* is indicative of hyperactivity of the hormone-producing organ, which is mostly caused by related components like as testosterone and estrogen as well as other factors including light, temperature stress, and nutrition [51].

In this study, Blood TSH levels were considerably high in the groups treated with microplastics. It is significant to remember that the first useful test for determining thyroid function is the measurement of TSH. Additional associated factors like T3 and T4 would be present in addition to the diagnosis of thyroid problems. Thyroid function in those who were exposed was evaluated in this study using estimates of T3 and T4, in addition to a very sensitive TSH test [53]. It was shown by Al-Shanti and Yassin [50] that there was a linear trend showing a correlation between higher TSH and long-term pesticide exposure.

The typical hematological and serological parameters are altered by microplastics, which are highly hazardous [25]. The present study showed a significant increase of TRBC and PCV in G2 while a significant increase of TWBC and MCV in G3. Hematological parameters suchlike Hb, PCV, and WBC are closely related to the circulatory system malfunction and with exposure to the environmental toxicants [54]. Sharma and Langer [55] have detected that cadmium leads to suppression of hemoglobin synthesis, decreasing the amount of PCV which disagrees with recent findings. According to the Debasmita *et al.* [56] elevation in WBC count might be resulted in response of immunological disorders. Overall, current findings coincide with the findings of Ali *et al.* [57].

Present research showed that CK-NAC, ASAT/GOT and Urea increased significantly due to microplastic ingestion. Following exposure to MPs, biochemical markers (including creatinine, uric acid, AST, ALT, cholesterol, total protein, and albumin) significantly increased in a dose-dependent manner. Our findings show that following exposure to MPs and/or pyrene, alterations in metabolic markers were seen in common gobies (*Pomatoschistus microps*) [58] as well as to MPs and/or nickel [59]. The immune system of the organism being interfered with, leading to cell damage, or the organism's

response to being exposed to microplastic could be the cause of the AST alterations. These enzymes seep into the body's circulatory fluid once cells is harmed, and it is widely acknowledged that an increase in these enzymes in the extracellular fluid is suggestive of even modest cellular damage. Likewise, it has been documented that tilapia (*Oreochromis niloticus*) serum had elevated AST and ALT activity following a 15-day exposure to MPs [60].

The nitrogen metabolite created by the animal body's breakdown of proteins serves as a defense against nitrogen toxicity and is known as urea. Urea is removed from the blood by glomeruli as it flows through Bowman's capsule, and the urine is the result [61]. Consequently, the glomerular filtration rate is determined by the concentration of urea in the proximal tubule of the nephrons. Variations in blood urea content could be a useful tool for evaluating nephron health [62]. According to the study's findings, turtles' glomerular filtration rate can be disturbed, and renal nephron damage can arise from MP exposure. According to research by Hamed *et al.*, [60], decreased renal function may have contributed to the rise in urea levels in the blood of tilapia (*O. niloticus*) exposed to MPs. Moreover, the impact of MPs on the primary metabolic pathways involved in the urea cycle may be the cause of elevated urea in the blood [63]. In present research microplastic leads to an increase in the level of CK-NAC previous research does not focus primarily on this parameter.

Conclusions

This study showed that microplastic ingestion altered the thermoregulation, digestive enzymes activity, hormonal activity as well as the hematology and serum biochemistry of *Gallus gallus domesticus* specifically in G3, which ingested the maximum quantity of microplastics. These modifications may lessen *Gallus'* net calorie intake from food. This may therefore reduce the amount of energy available for body growth, which may influence population dynamics and individual fitness. The lower growth reported in the chickens that have ingested microplastics may be the result of changes in energy flux caused by microplastic. Additional research to carry out the evaluation of possible toxicity of microplastic on birds is strongly recommended, it also will be crucial to understand the process by which microplastics affect the terrestrial and aquatic life as well as their role as micro-pollutant in the environment.

Conflicts of interest

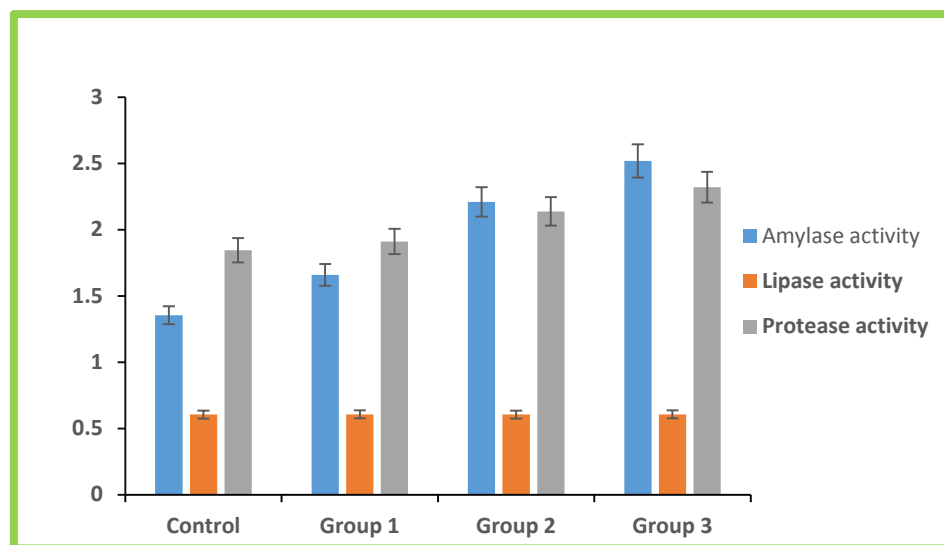
The authors declare that they have no conflict

TABLE 1. Effect of microplastic ingestion on body weight gain (gm) in chickens

Days of trial	Control	Group-1	Group-2	Group-3
1-14	31.25±23.94	25±20.41	30±5	18.75±12.5
15-28	56.25±12.5	33.75±17.5	38.33±10.41	31.25±12.5
29-42	63.75±11.09	31.25±23.94	51.67±2.89	33.75±26.89
43-56	70±11.55	71.25±14.36	65±8.67	11.25±40.90
57-70	80±10.25	62.5±33.04	66.67±5.78	58.75±15.48
71-84	100±4.2	115±17.32	80±10	72.5±14.43
85-98	107.5±9.57	115±26.46	96.67±5.78	83.75±17.02
99-112	135±12.91	110±33.67	123.33±5.78	91.25±18.23

TABLE 2. Effect of microplastic ingestion on chickens' body temperature (°F)

Days of trial	Control	Group-1	Group-2	Group-3
1-14	104.33±0.15	103.85±0.35	104.73±0.39	104.1±0.22
15-28	104.96±0.21	104.6±0.47	105.76±0.79	105.13±0.39
29-42	106.63±0.15	105.35±0.21	106.2±0.42	106.23±0.34
43-56	106.27±0.21	106.13±0.17	107.13±0.33	106.33±0.35
57-70	107.13±0.35	106.98±0.29	107.28±0.48	107.25±0.62
71-84	107.5±0.3	107±0.81	107.93±0.21	107.85±0.29
85-98	107.33±0.15	107.9±0.61	108.68±0.17	108.38±0.51
99-112	107.37±0.15	107.93±0.83	108.35±0.13	108.95±0.48

**Fig. 1. Activity of enzymes with microplastic ingestion in chickens**

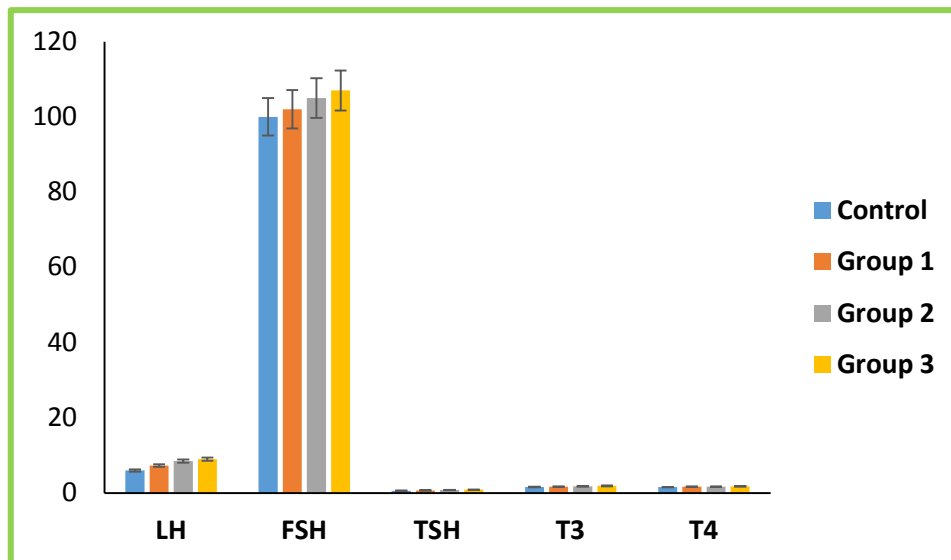


Fig. 2. Various hormonal activities with microplastic ingestion in chickens

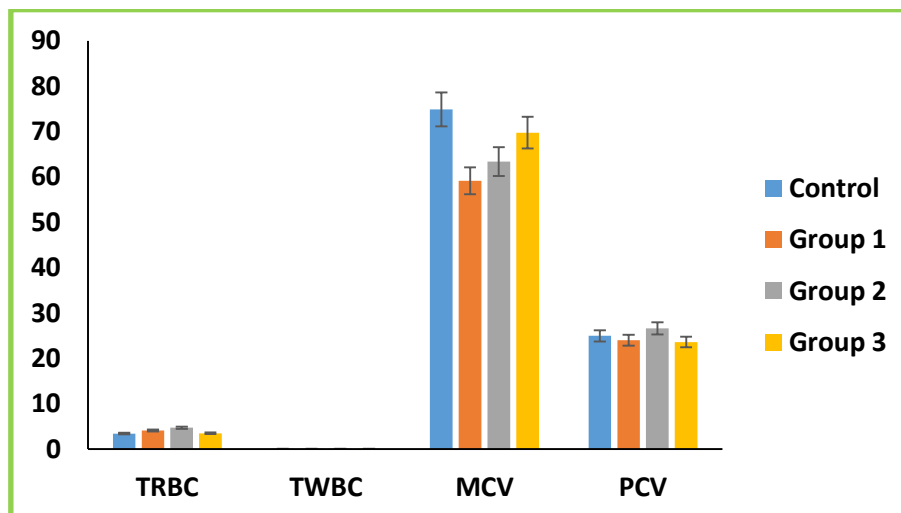


Fig. 3. Various hematological indices with microplastic ingestion in chicken

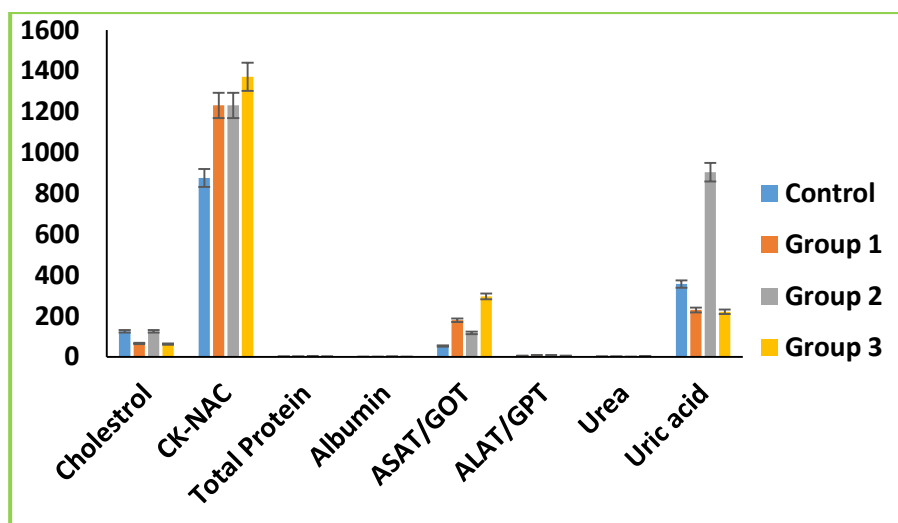


Fig. 4. Serological parameters with microplastic ingestion in chickens

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تأثير ابتلاع البلاستيك الدقيق على الإنزيمات الهضمية، والهرمونات، أمراض الدم والكيمياء الحيوية في الدم لجالوس جالوس دومينيكوس

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الخلاصة

تهدف الدراسة الحالية إلى تحديد كيفية تأثير المواد البلاستيكية الدقيقة على معدل نمو الدجاج المنزلي، ودرجة حرارة الجسم، والإنزيمات الهاضمة، ومستويات الهرمونات، وأمراض الدم، والكيمياء الحيوية في المصل. في سجل النمو ودرجة حرارة الجسم، كانت الزيادة في وزن جسم الطائر هي الحد الأدنى لدرجة حرارة الجسم وكانت الحد الأقصى في المجموعة الثالثة (G3) مقارنة بالطيور الأخرى. كانت المعلمات الدموية، أي إجمالي عدد خلايا الدم البيضاء (TWBC) ومتوسط حجم الكريات (MCV) هي الأعلى في G3 بينما كان إجمالي عدد خلايا الدم الحمراء (TRBC) وحجم الخلايا المعبأة (PCV) الأعلى في G2. أظهر الكرياتينين كيناز (CK-NAC)، وناقلة أمين الأسبارتات (ASAT)، واليوريا أقصى قيمة في G3 بينما كان مستوى الكوليسترول والبروتين الكلي والألبومين والأنين أمينوترانسفيراز (ALAT) وحمض البوليك أعلى في G2. لوحظت زيادة معنوية في مستوى الأميليز وإنزيمات البروتياز الكلي وجميع الهرمونات مثل الهرمون اللوتيني (LH)، الهرمون المنبه للغدة الدرقية (TSH)، الهرمون المنبه للجريب (FSH)، ثلاثي يودوثيرونين (T3)، رباعي يودوثيرونين (T4) في المجموعة الثالثة. بينما يبقى إنتاج إنزيم الليباز دون تغيير. بشكل عام، أظهرت المجموعات التجريبية مستويات هرمونية أعلى وإنتاجاً إنزيمياً مع انخفاض في زيادة وزن الجسم مما قد يشير إلى أن الملوثات الغذائية تؤدي في النهاية إلى خفض صافي استهلاك الدجاج من الطاقة من الطعام الذي يستهلكونه واحتياجات الطاقة لديهم.

الكلمات المفتاحية: البلاستيك الدقيق، الدجاج، درجة حرارة الجسم، أداء النمو، المستويات الهرمونية، الإنزيم الهضمي، الكيمياء الحيوية في الدم، أمراض الدم