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EFFECTS OF GREYWATER MICROPARTICLES ON THE PHYSIOLOGY OF *GALLUS GALLUS DOMESTICUS*

A. Khadim *[1], M. Arshad *[1], W. Ijaz [1], S. Jaffar [1], T. Ruby [1], A. A. Khan [1] and A. Ashraf [2]

[1] Institute of Zoology, Bahauddin Zakariya University, Multan, Pakistan.
[2] Department of Zoology, The Women University, Multan, Pakistan.

*** Correspondence:**
Khadimayesha0@gmail.com;
maryamarshad616@gmail.com
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ABSTRACT. *Greywater-derived microparticles may affect animal health, but their effects on vertebrate physiology remain poorly understood. This study investigated the effects of greywater microparticles on immune responses, digestive enzyme activity, nutritional composition, and meat composition in Gallus gallus domesticus. Composite laundry greywater collected from residential households was evaporated to obtain a dry residue, which was mechanically ground to produce the heterogeneous particulate material referred to as greywater microparticles. Thirty-six chickens were divided into a control group and three experimental groups receiving greywater microparticles at 300, 600, and 900 mg/kg body weight/day for 12 weeks. Immune parameters were assessed after 2 and 12 weeks, while other parameters were evaluated after 12 weeks. Exposure was associated with reduced IgA, IgY, and ceruloplasmin concentrations, whereas IL-2 and TNF increased. IL-6 and CRP showed time-dependent responses. Amylase, lipase, and protease activities increased following exposure. Crude protein, dry matter, and ether extract decreased, whereas nitrogen-free extract increased. In meat, moisture, crude protein, lipid, ash contents and gross energy decreased significantly with increasing exposure to greywater microparticles, whereas gross energy showed a non-linear response. Overall, greywater microparticles exposure was associated with alterations in immune, digestive, nutritional, and meat parameters.*

Keywords:
Greywater Microparticles; Chickens; Immune Response; Digestive Enzymes

INTRODUCTION

Disposing greywater into the environment is a common practice in developing countries. Countries facing water scarcity tend to reuse greywater for irrigational purposes (Budeli & Sibali, 2025). Untreated greywater has pathogens and chemical compounds that may pose risk to environmental quality and living organisms (Neta & Friedler, 2025). Many studies have investigated the potential contamination of water bodies following greywater discharge because some chemical contaminants may persist in the environment or undergo transformation into potentially harmful products (Shuai *et al*., 2023). Moreover, microbial contamination should not be overlooked since microbes have the ability to survive and propagate. They can reach the infective dose and become hazardous for humans and animals (Petousi *et*

al., 2023). A wide range of opportunistic pathogens, which are able to cause primary and secondary infections in organisms including humans are widely reported to be present in greywater (Arabeyyat, 2024).

Greywater generation varies according to household practices, water availability, and geographical setting, with reported estimates reaching approximately 200 litres per person per day in some settings (Abusam *et al*., 2025). Laundry greywater may contain surfactants, detergents, bleaching agents, suspended solids, oils, and other household-derived contaminants (Jena *et al*., 2023). Beauty and cosmetic products, hair and skin cells, particles of sand and clay, chemicals from toothpastes, soaps, and shaving creams, all constitute in wastewater discharged from bathrooms (Elhegazy & Eid, 2020).

Moreover, greywater may provide suitable conditions for infectious pathogens for survival and growth (Shuai *et al*., 2023). Microbial contaminants in greywater may pose health risks depending on the route and extent of exposure, particularly when greywater is untreated or inadequately treated (Mahmoud *et al*., 2026).

Water is an essential component of poultry production and can influence digestion, physiological function, health, and immune status. The quality of water available to poultry is affected by its physicochemical and microbiological characteristics, and suboptimal water quality may influence physiological and immune responses in birds. Recent evidence has further emphasized the importance of water quality in poultry health and immune function, indicating that water can represent an important route through which environmental factors may affect poultry production and disease resistance (Wlaźlak *et al*., 2023; Farooq *et al*., 2026). Therefore, exposure of poultry to contaminated or poor quality water and water derived materials warrants consideration as a potential source of physiological stress.

In an experiment, chickens fed with acidified drinking water had an increased activity of the lipase enzyme in the gut (Hamid *et al*., 2018). The gastrointestinal tract represents an important route of exposure to ingested environmental contaminants, where interactions with intestinal tissues may influence local and systemic physiological and immune responses (Awad *et al*., 2017). In chicken farms, water quality has detrimental effects on broiler health, which negatively affects body weight as well as immunity (El Miniawy *et al*., 2017).

Poultry farms use water from wells, streams, ponds, or from municipal sources. Low quality of water can affect water consumption by chickens (El Miniawy *et al*, 2017; Arsenopoulos *et al*., 2026). Poor health due to infectious diseases is directly responsible for adverse economic impacts on poultry (Hafez & Attia, 2020).

Various factors, such as, genetics, healthcare, managing practices, and type of housing, all play a key role in poultry performance and production, but water quality is a main factor and is often neglected the most (Sheikh, 2019). Water for drinking plays fundamental role in spread and transmission of common poultry diseases, which can be bacterial, viral and protozoan diseases (Coelho *et al*., 2026).

Although the occurrence and potential environmental and health risks of contaminants in greywater have been increasingly investigated, emerging contaminants and their potential risks remain important areas of research (Mustafa *et al*., 2025; Hamidi, 2025). However, comparatively less attention has been given to the biological effects of the concentrated particulate residue obtained after drying and processing greywater (González *et al*., 2025). Such residues may contain a heterogeneous mixture of organic and inorganic constituents derived from household activities and may represent a concentrated form of exposure. Therefore, the physiological responses of poultry following dietary exposure to greywater-derived particulate residues remain insufficiently characterized. Investigating the effects of greywater microparticles on immune responses, digestive enzyme activity, nutritional composition, and meat composition may provide further insight into the potential biological consequences of exposure to these residues.

This study investigated the effects of greywater microparticles on immune responses, activity of digestive enzymes, nutritional composition and proximate meat composition of chickens. Although greywater is not suitable for direct consumption, the use of poor quality water in poultry production may adversely affect animal health, highlighting the importance of investigating potential physiological consequences of exposure to greywater-derived microparticles.

MATERIALS AND METHODS

Collection and Preparation of Greywater Derived Microparticles

Laundry greywater was collected from 15 residential households in Multan, Punjab, Pakistan. Approximately 1 L of laundry greywater was collected from each household in sterile containers and transported to the laboratory at Bahauddin Zakariya University, Multan. The individual samples were pooled to obtain a single composite laundry-greywater sample.

In the present study, the term greywater microparticles refers specifically to the dried and mechanically ground particulate residue obtained from the composite laundry greywater. The composite greywater sample was subjected to controlled evaporation under laboratory conditions until a visible dry residue was obtained. The resulting residue was subsequently dried until a constant mass was achieved. The dried material was mechanically ground to obtain a homogeneous particulate preparation, which was subsequently used for experimental administration to the chickens.

The prepared greywater microparticles were characterized for dry-residue yield, moisture content, ash content, particle size, microscopic morphology, and elemental composition. The dry-residue yield was 2.5 g/L of the original composite greywater. The moisture and ash contents of the dried material were 4.2% and 41.3%, respectively. Particle size was determined by microscopic examination, which showed a particle-size range of approximately 20–130 µm, with a mean particle size of approximately 74 µm. Microscopic examination revealed a heterogeneous morphology, predominantly consisting of irregular and angular particles, with occasional rounded aggregates and smaller particles adhering to larger particles.

The elemental composition of the dried greywater microparticles preparation was determined by atomic absorption spectrophotometry. The measured concentrations were 8,420 mg/kg calcium (Ca), 2,180 mg/kg magnesium (Mg), 214 mg/kg iron (Fe), 5.8 mg/kg chromium (Cr), 2.3 mg/kg lead (Pb), and 0.16 mg/kg cadmium (Cd). These measurements provided the physicochemical and elemental characterization of the greywater microparticles preparation used in the exposure experiment. Table 1 enlists the physicochemical characteristics of prepared greywater microparticles.

Table 1. Physicochemical properties and elemental concentration of greywater microparticles

Parameter / Element	Concentration / Value
Calcium (Ca)	8,420 mg/kg
Magnesium (Mg)	2,180 mg/kg
Iron (Fe)	214 mg/kg
Chromium (Cr)	5.8 mg/kg
Lead (Pb)	2.3 mg/kg
Cadmium (Cd)	0.16 mg/kg
Dry-residue yield	2.5 g/L
Moisture content	4.2%
Ash content	41.3%
Particle-size range	20–130 μm

Experimental Animals and Exposure Design

Thirty-six female *Gallus gallus domesticus* (Misri) chickens, approximately four months old and with initial body weights ranging from 400 to 500 g, were used in the experiment. The chickens were maintained in the animal-house facility of the Biological Garden, Bahauddin Zakariya University, Multan, under routine animal-house management conditions, including appropriate temperature, humidity, ventilation, and photoperiod. The chickens were allowed to acclimatize to the animal-house environment for one week before initiation of the treatment trial.

Following acclimatization, the chickens were allocated into four groups, comprising one control group and three experimental groups (G1, G2, and G3), with nine chickens in each group. The experimental groups received greywater microparticles at doses of 300, 600, and 900 mg/kg body weight/day (equivalent to 0.03%, 0.06% and 0.09% of body weight) for G1, G2, and G3, respectively. The greywater microparticles were administered once daily for 12 weeks. The required quantity of greywater microparticles was incorporated into partially cooked wheat roti and manually administered to the chickens.

The same feeding procedure was followed for all groups. All chickens received the same formulated poultry feed, while the control group received the feed without greywater microparticles supplementation. The chickens were routinely observed for behavioral changes and signs of disease throughout the experimental period and were immunized against prevalent poultry diseases.

Ethical Considerations

The study involved chickens maintained in institutional animal house facility of Bahauddin Zakariya University, Multan. No separate animal ethics approval number was assigned to this study.

Collection of Blood Samples

Blood samples were collected from the wing veins of chickens after 2 and 12 weeks of exposure to determine the concentrations of immune-related parameters. Samples were collected in EDTA containing tubes and centrifuged to separate the plasma, which was used for subsequent analysis of the immune parameters.

Assessment of Immune Parameters

To check the effect of greywater microparticles on immune response of chickens, immune parameters, IgA, IgY, IL-2, IL-6, TNF, Cp, and CRP were assessed using commercially available enzyme linked

immunosorbent assay (ELISA) kits while following manufacturer's instructions. Albumin was assessed by Bromocresol green technique (BCG).

Crop Removal and Enzyme Extraction

Crop samples were carefully collected from the chickens and rinsed with Tris-HCl buffer to remove external debris. The samples were wrapped in aluminium foil and stored frozen until enzyme extraction. Enzymes were extracted from the crop samples using Tris-HCl buffer. Starch, casein, and olive oil were used as substrates for the determination of amylase, protease, and lipase activities, respectively. Amylase activity was assessed by measuring the production of reducing sugars, lipase activity by measuring the release of free fatty acids, and protease activity by measuring the products of protein hydrolysis.

Analysis of Nutritional Composition

The nutritional composition of crop contents collected from the experimental chickens was assessed after 12 weeks of exposure to greywater microparticles. The parameters evaluated included dry matter, crude protein, ether extract, ash, and nitrogen free extract (NFE). Dry matter was determined by oven drying at 135°C for 4 h. Crude protein was determined using the Kjeldahl method, with nitrogen content multiplied by a conversion factor of 6.25. Ether extract was determined using Soxhlet extraction, while NFE was calculated by difference.

DM (%) = [Sample weight after drying / Sample weight before drying] × 100

NFE % = 100 − [Moisture % + Crude protein % + Ether extract % + Ash %]

Analysis of Meat Composition

Moisture content of meat samples was analysed using the Karl Fischer titration method (MacLeod, 1991). Crude protein was quantified using the Kjeldahl method, with crude protein calculated as total nitrogen multiplied by 6.25 (Goyal *et al*., 2022). The Kjeldahl method consisted of digestion, distillation, and titration. Kjeldahl nitrogen (%) was calculated as:

Kjeldahl nitrogen % = $[(V_s - V^B) \times M \times 14.01] / (W \times 10)$

Where V_s is the volume (mL) of standardized acid to titrate the test sample, V^B is the volume (mL) of standardized acid to titrate the reagent blank, M is the molarity of the standard HCl, the atomic weight of nitrogen, and W is the weight (g) of the standard. Crude protein (%) was determined as Kjeldahl nitrogen (%) × F, where F = 6.25. Lipid content was measured by repeated extraction of the sample with diethyl ether (boiling at 40–60°C) using a Soxhlet apparatus.

Determination of Gross Energy

Gross energy (GE) was calculated by a bomb calorimeter. Samples were dried prior to analysis, and the bomb calorimeter was calibrated using benzoic acid as standard. The gross energy content of the samples was analyzed following previously described procedures (Sharma *et al*., 2021; Adeola & Ileleji, 2009).

Determination of Total Ash

Ash content was evaluated by the dry-ashing method according to AOAC Official Method 942.05. About 2g sample was placed into a pre-weighed porcelain crucible which was then placed in a muffle

furnace pre-heated to 600°C. After 2 h, the samples were transferred to a desiccator, cooled, and weighed. Ash content was expressed as a percentage of the original sample weight (Thiex *et al*., 2012).

Statistical Analysis

Data obtained for immune parameters, digestive enzyme activities, nutritional composition, and meat composition were analysed using IBM SPSS Statistics. For immune parameters assessed at 2 and 12 weeks, two way analysis of variance (ANOVA) was used to evaluate the effects of treatment group, sampling time, and their interaction. For parameters assessed at 12 weeks only, differences among treatment groups were evaluated using one way ANOVA. Where significant differences were detected, Tukey's honestly significant difference post hoc test was used for multiple comparisons. Descriptive statistics were calculated, and data are presented as mean ± standard deviation (SD). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Parameters of Immune System

To check the effects of greywater microparticles on immune responses of chickens, blood samples were taken twice, after 2 weeks of trial and after 12 weeks. Consequently, results were also recorded twice. All of the 36 chickens, divided into 4 groups (one control), each consisting of 9 chickens, were subjected to descriptive analysis (Mean ± SD) of albumin, IgA, IgY, Cp, IL-2, IL-6, TNF and CRP.

The amounts of immune parameters were measured after 2 weeks. It showed that the amounts of greywater microparticles and albumin concentration in three groups remain stable with P = 0.108. As the amount of greywater microparticles was increased from G1 to G3 group, the concentration of IgA and IgY antibodies in subsequent groups declined significantly with P = 0.002 and P = 0.014 as compared to the control group. There was steady decrease in the concentration Cp protein with P = 0.020. The amount of IL-2 was significantly increased in treated groups having P = 0.001. Conversely, IL-6 concentrations were decreased with P = 0.002. A positive relation was seen between the amounts of TNF and greywater particles with P value < 0.05, p = 0.004. The amount CRP was increased with P = 0.001. The results of the statistical analysis of blood samples taken after two weeks are listed in Table 2 and graphical representation in Fig. 1.

Table 2. Mean ± SD and P values of immune parameters of chickens measured after two week of the treatment trial

Parameters	Control	Group 1	Group 2	Group 3	P value*
Albumin (g/L)	7.02±0.25	6.52±0.53	5.03±0.71	5.25±0.32	0.108
IgA (ug/mL)	130.3±3.26	125.5±1.60	118.7±1.21	112.2±3.06	0.002**
IgY (ug/mL)	1205.2±94	1012.6±81	985.5±7.08	825.3±13	0.014*
CP (U/L)	5.04±1.54	4.61±0.42	4.28±0.78	4.01±0.42	0.020*
IL-2 (PG/mL)	503.5±2.35	678.9±18	854.3±12	905.4±24.0	0.001**
IL-6 (ng/L)	115.6±9.0	110.3±4.0	92.8±9.83	88.5±5.87	0.002**
TNF (ng/L)	160.8±6.0	142.3±7.0	180±9.80	192.5±28	0.004**
CRP (mg/dL)	1.20±0.44	1.14±0.28	1.2±0.2	1.33±0.20	0.001**

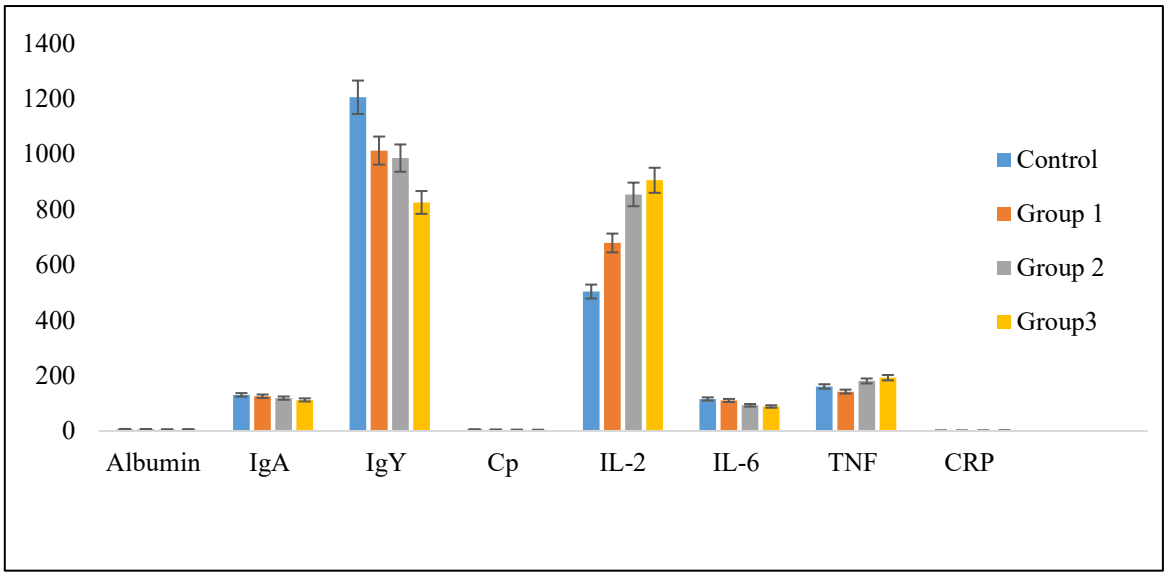

Fig 1. Average amounts of immune indices in control group and greywater microparticles treated groups of chickens after 2 weeks of trial. Values are presented as mean ± SD

The blood samples from all of the 36 chickens were subjected to analysis after 12 weeks of trial. The amounts of albumin remained almost same in treated groups with P = 0.206. The concentrations of IgA and IgY were decreased in treated groups having P = 0.05 and 0.001 respectively. Likewise, the amounts of Cp was significantly recorded to be lower than control group with P= 0.001. IL-2 concentrations of chickens in treatment groups was increased with P = 0.023. The amounts of IL-6 was not significantly changed with P = 0.326. The amounts of TNF were increased showing P = 0.005. The amounts of CRP was decreased in treated groups with P= 0.001. Table 3 shows the mean ± SD and P values of immune indices. Figure 2 represents the amounts of all parameters of treated and control group.

Table 3. Mean ± SD and P values of immune parameters in control and greywater microparticles treated groups of chickens measured after 12 weeks of trial

Parameters	Control	Group 1	Group 2	Group 3	P value*
Albumin (g/L)	16.02±1.58	22.22±3.81	18.73±5.81	24.35±6.54	0.206
IgA (ug/mL)	260.3±34.6	225.6±12.2	145.6±7.43	237.3±5.98	0.05
IgY (ug/mL)	605.2±12.9	525.3±47.8	475.1±27.8	554.8±14.1	0.001**
CP (U/L)	8.04±2.71	5.75±2.52	5.18±1.37	5.98±1.21	0.001**
IL-2 (PG/mL)	103.7±8.30	178.4±15.9	105.3±8.79	186.2±10.0	0.023*
IL-6 (ng/L)	105.6±5.95	90.3±10.10	112.8±3.67	120.3±5.60	0.326
TNF (ng/L)	260.8±21.2	197.2±22.3	292.2±54.2	295.6±37.2	0.005**
CRP (mg/dL)	1.24±0.13	1.08±0.10	1.06 ± 0.38	1.17±0.19	0.001**

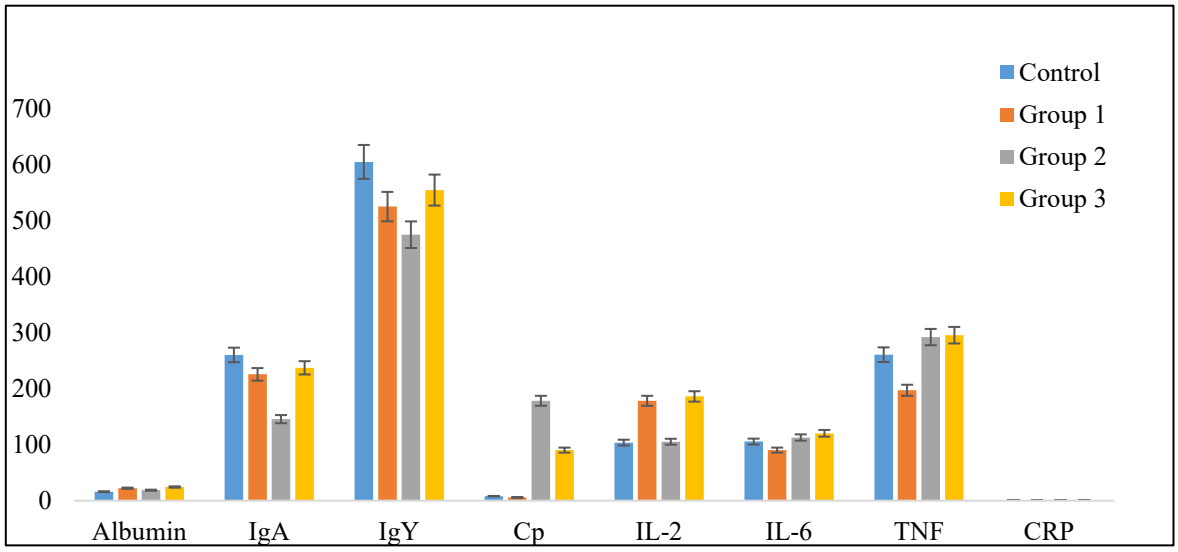

Fig 2. Average amounts of immune indices in control group and treated groups after 12 weeks of trial. Values are represented as mean±SD

Enzyme Activities and Nutritional Composition

The activity of amylase was increased in the treatment group of chickens with highly significant P= 0.001. The activity of lipase in subsequent treatment groups G1, G2 and G3 was also increased, P= 0.001. The activity of protease was also increased P = <0.001. Table 4 and Figure 3 shows the activity (Mean ± SD) of digestive enzymes.

Table 4. Mean ± SD and P values of parameters of Nutritional composition and Digestive enzymes' activity in control and greywater microparticles treated groups of chickens

Parameters	Control	Group 1	Group 2	Group 3	P value*
Amylase	8.06±0.91	9.83±1.25	11.07±0.84	10.33±1.03	0.001**
Lipase	9.12±0.47	10.3±0.53	12.39±0.7	13.71±2.17	0.001**
Protease	28.3±1.51	30.5±2.16	39.5±1.51	40.2±0.98	<0.001**
Dry matter	95.2±1.85	82.1±1.65	77.5±2.91	72.5±2.25	0.001**
Crude Protein	98.2±2.43	102.5±2.16	99.3±2.09	91.6±1.35	0.002**
Ether extract	83.5±2.18	57.5±2.2	61.3±1.18	64.8±1.76	0.007**
Nitrogen free extract	93.6±1.77	98.2±2.45	101.3±1.99	103.4±1.44	0.032*

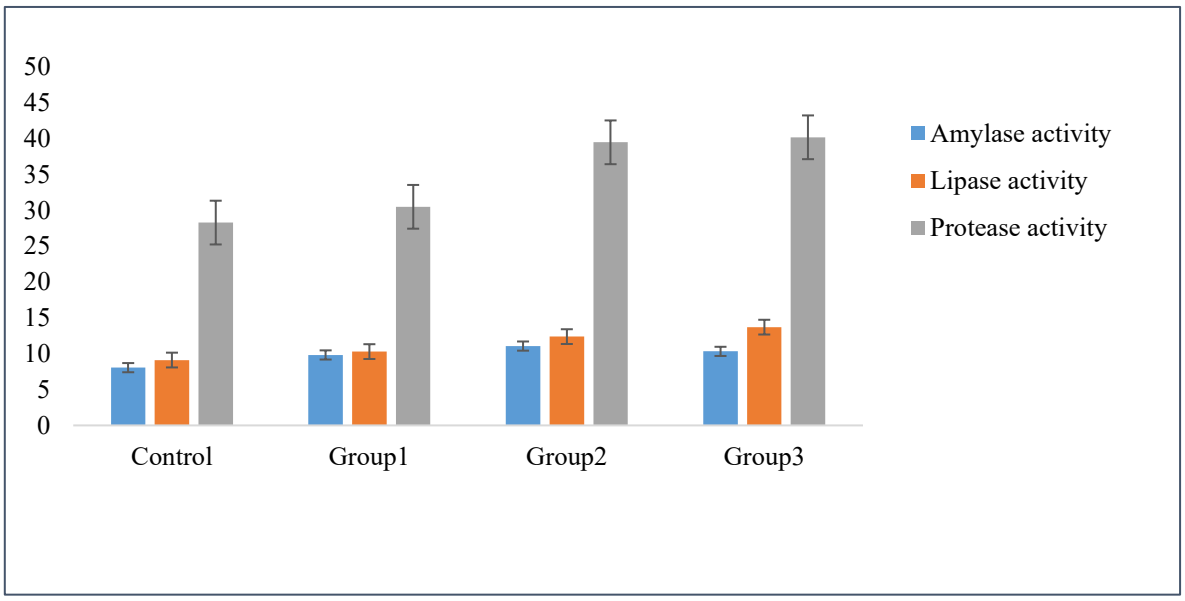

Fig 3. Activity of digestive enzymes in control and experimental groups of chickens treated with greywater microparticles after treatment trial of 12 weeks. Values are represented as mean±SD

The concentrations of dry matter and crude protein were significantly decreased in treated groups with P = 0.001 and P= 0.002 respectively. Similarly, the concentrations of ether extract were decreased in treated groups than in control groups with P = 0.007. The amounts of nitrogen free extract was not changed with P = 0.032. Table 3 shows the average amounts of nutritional composition parameters in treated groups of chickens and control group. Table 4 and Figure 4 represents graphical explanation of the amounts of nutritional composition parameters in control groups and treated groups.

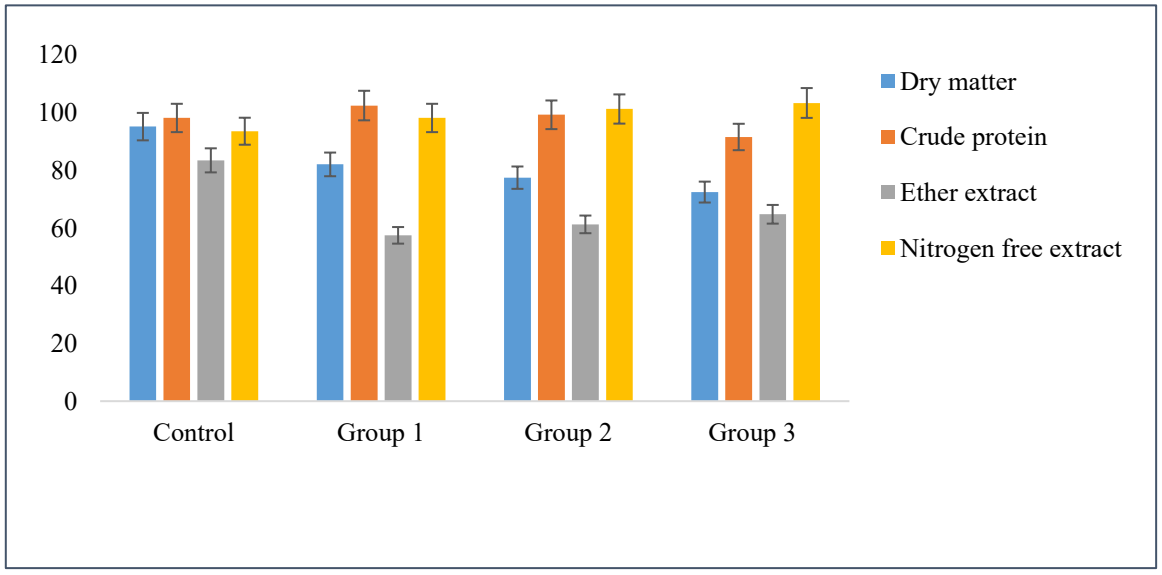

Fig 4: Amounts of parameters of nutritional composition in control and treated groups of chickens with greywater microparticles. Values are represented as mean±SD

Parameters of Meat Composition

The amount of moisture content in the meat samples was significantly lowered in treated chickens than in control group, P= 0.0001. The amounts of crude protein was decreased in meat samples of treatment groups, P= 0.0002. The content of lipids in treated groups was less than in control group with P value of 0.0076. The amounts of total ash and gross energy (kcal) were decreased in treatment groups with P= 0.0312 and P= 0.0001. Table 5 and graphical representation in Figure 5 show the parameters of proximate meat composition in control and treated groups.

Table 5. Mean ± Standard deviation of parameters of meat composition in chickens of control group and treated group measured after treatment trial

Parameters	Control	Group 1	Group 2	Group 3	P value*
Moisture content	80.20±1.47	72.12±1.38	68.58±1.37	63.50±1.80	0.0001***
Crude Protein	32.15±1.56	24.30±1.52	20.08±1.54	18.54±2.03	0.0002***
Lipids	1.53±0.18	1.57±0.18	1.52±0.17	1.34±0.20	0.0076**
Total ash	1.33±0.21	1.34±0.20	1.25±0.15	1.17±0.14	0.0312*
Gross energy	4089±156	4595±167	4262±144	4078±161.1	0.0001***

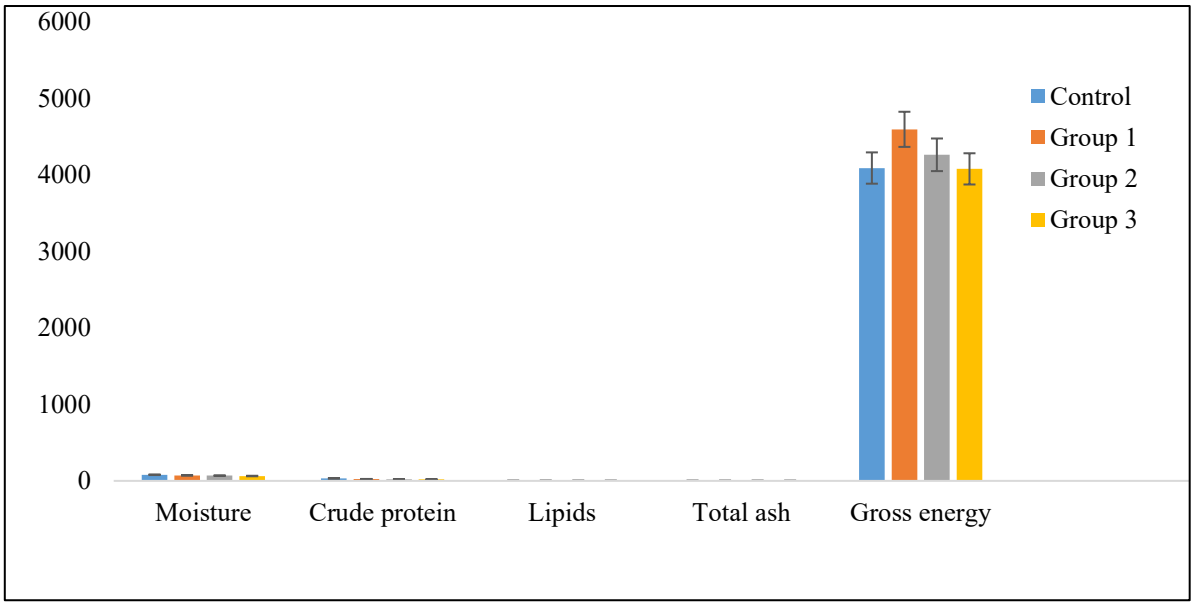

Fig 5. Parameters of proximate meat composition in chickens of control and treated groups measured after treatment trial. Values are represented as mean±SD

DISCUSSION

Exposure to greywater microparticles was associated with alterations in several immune related parameters during the experimental period. The measured parameters included immunoglobulins IgA and IgY, cytokines IL-2, IL-6 and TNF, and other immune- and inflammation-related indicators such as ceruloplasmin and C-reactive protein. These parameters are involved in different aspects of avian immune and inflammatory responses (Kou *et al*., 2019); however, changes in their concentrations alone do not establish a specific underlying mechanism. Greywater-derived particulate residues may contain a heterogeneous mixture of organic and inorganic constituents originating from household activities, including surfactants, detergents, suspended solids, and other contaminants (Jena *et al*., 2023; *Budeli & Sibali*, 2025). Therefore, the observed changes in immune-related parameters may reflect the biological response to the combined constituents present in the greywater microparticles rather than the effect of a single contaminant. The elemental characterization of the greywater microparticles in the present study demonstrated the presence of Ca, Mg, Fe, Cr, Pb, and Cd, indicating that the particulate residue contained both major mineral elements and trace elements that contributed to its overall composition.

In the present study, albumin concentrations did not differ significantly between the control and treated groups after 2 weeks or 12 weeks of exposure ($P > 0.05$). Albumin is generally considered a negative acute-phase protein in birds, and its concentration may decrease during an acute-phase response, whereas other acute-phase proteins increase during inflammatory or infectious challenges (Cray, 2012; Murata *et al*., 2004). However, the absence of a significant change in albumin concentration in the present study suggests that greywater microparticles exposure, under the conditions investigated, did not produce a detectable alteration in this parameter. This finding should be interpreted cautiously because albumin concentration can be influenced by several physiological and nutritional factors and, by itself, does not establish the presence or absence of an inflammatory response.

The present study demonstrated significant reductions in IgA and IgY concentrations in the treated groups compared with the control group after both 2 and 12 weeks of exposure. The concentrations of both immunoglobulins generally decreased across the treatment groups, particularly at higher doses. IgA is an important component of mucosal immunity in chickens, whereas IgY is the major circulating immunoglobulin involved in systemic humoral immune responses (Wlaźlak *et al*., 2023). Alterations in circulating immunoglobulin concentrations can therefore indicate changes in humoral immune status, although they do not by themselves identify the underlying cause or mechanism. Previous studies of environmental particulate exposure in chickens have also reported alterations in physiological and immune-related responses following exposure to particulate contaminants. For example, experimental exposure to microplastics has been associated with adverse physiological effects and changes in immune-related tissues in chickens (Li *et al*., 2023; Guo *et al*., 2024). However, the greywater microparticles investigated in the present study were not purified microplastics but heterogeneous particulate residues obtained from greywater; therefore, direct comparison with studies involving defined plastic particles should be made cautiously. The reductions in IgA and IgY observed here consequently indicate an alteration in humoral immune-related parameters following GWMP exposure, but the specific mechanism responsible for these changes cannot be determined from the present measurements alone.

Findings of this research indicated that levels of protein ceroluplasmin are decreased considerably ($P < 0.05$) after 2 weeks of commencement of greywater microparticles and after 12 weeks of trial. Ceruloplasmin is an acute-phase protein involved in copper transport and other physiological functions and is generally classified as a positive acute-phase protein in birds (Murata *et al*., 2004). The decrease observed in the present study therefore represents an alteration in an acute-phase-related parameter; however, it should not be interpreted as direct evidence of either increased or decreased inflammation.

The present study showed that exposure to greywater microparticles altered the concentrations of several cytokines. IL-2 concentrations were significantly higher in the treated groups after both 2 and 12 weeks of exposure, whereas IL-6 concentrations were significantly lower after 2 weeks and did not differ significantly among groups after 12 weeks. In contrast, TNF concentrations were significantly higher in the treated groups at both sampling times. IL-2, IL-6 and TNF are important mediators involved in the regulation of immune responses in chickens, and their concentrations can vary according to the nature and duration of an immune or physiological challenge (Wlaźlak *et al*., 2023). Recent studies have demonstrated that particulate exposure can also alter inflammatory responses in chickens. Fine particulate matter exposure, for example, was associated with changes in IL-6 and TNF-α expression in broilers, while experimental exposure to polystyrene microplastics produced inflammatory responses in chicken immune tissues (Li *et al*., 2023; Zhou *et al*., 2024). These findings provide contextual support for the possibility that particulate exposure can influence immune-related responses in poultry; however, the materials and exposure conditions differed substantially from those used in the present study.

C-reactive protein (CRP) concentrations also showed a time-dependent pattern following exposure to greywater microparticles. After 2 weeks, CRP concentrations were higher in the treated groups, whereas after 12 weeks, the treated groups showed lower CRP concentrations than the control group. CRP is an acute-phase protein involved in innate immune responses and can change in response to infection, tissue injury, and other physiological challenges; however, the magnitude and pattern of acute-phase protein responses vary considerably among animal species, including birds (Nawaz *et al*., 2021; O'Reilly & Eckersall, 2014)). Therefore, the increase observed at 2 weeks may indicate an early alteration in an acute-phase-related response, whereas the lower concentrations observed after 12 weeks may reflect a different physiological state following prolonged exposure. Nevertheless, these changes should not be interpreted as definitive evidence of either acute or chronic inflammation because CRP was assessed together with other parameters and no direct measurements of inflammatory pathways.

In the present study, the activity of amylase was significantly increased in all experimental groups (G1, G2 and G3) compared with the control group, while lipase and protease activities also increased with increasing greywater microparticles dose. Similar alterations in digestive enzyme activity have been reported following dietary supplementation with butyrate-loaded clinoptilolite in broiler chickens (Wu *et al*., 2016) and mixed organic acids, which increased pancreatic amylase activity (Ma *et al*., 2021). Al-Khalaifah *et al*. (2020) also reported that dietary treatment with fermented or enzyme-treated brewer's grains upregulated genes related to digestive enzymes in broilers, indicating that dietary composition can influence digestive function. Increased digestive enzyme activity has also been observed following exposure to or treatment against dietary toxicants; for example, Marchioro *et al*. (2013) reported alterations in pancreatic digestive enzyme activity following aflatoxin exposure, while Kairov *et al*. (2020) observed increased protease and amylase activities in broilers receiving an antioxidant during T-2 toxin exposure. Recent studies similarly demonstrate that dietary interventions can modify amylase, lipase and protease activities in broilers (Yaqoob *et al*., 2022; Zhou *et al*., 2023). Therefore, the increased enzyme activities observed following exposure may reflect an alteration or adaptive response of the digestive system to the dietary treatment; however, increased enzyme activity should not necessarily be interpreted as improved digestion or nutrient utilization. The present study did not directly measure nutrient digestibility or the mechanisms regulating enzyme secretion, and therefore the specific basis for the observed increase in digestive enzyme activities cannot be established.

Dry matter concentration was significantly decreased in the treatment groups compared with the control group. This finding differs from the results reported by Mutlu *et al*. (2021), who found that dietary quercetin supplementation had no significant effect on dry matter digestibility in quails. Differences between these findings may be related to the nature and composition of the dietary treatments and the experimental conditions. Nutrient composition and digestibility are also influenced by the relative proportions of dietary components, and changes in one component can affect the calculated proportion of others when values are expressed on a percentage basis (Sundu *et al*., 2019). Therefore, the lower dry matter concentration observed in the present study may reflect an alteration in the overall composition of the dietary material following exposure however, it should not be interpreted directly as reduced dry matter digestibility because digestibility was not measured in the present study.

In the present study, meat moisture, crude protein, lipid and ash contents generally decreased with increasing greywater microparticles concentration, although gross energy showed a non-linear response, increasing in G1 and G2 and decreasing in G3. Similar reductions in moisture, crude protein and lipid contents have been reported in chickens receiving dietary carrageenan (Jameel *et al*., 2024), although differences in the dietary material and experimental conditions should be considered. Li *et al*. (2016) also reported that increasing dietary phosphorus levels could alter breast meat composition, particularly moisture content, demonstrating that dietary constituents can influence meat characteristics. In addition, Verma *et al*. (2002) reported reductions in metabolizable energy in chickens exposed to dietary aflatoxins and ochratoxins. However, these previous studies involved defined dietary additives or mycotoxins, whereas the greywater microparticles used in the present study were heterogeneous particulate residues obtained from greywater. Therefore, the changes observed in meat composition and gross energy indicate an association with greywater microparticles exposure but the specific mechanisms responsible for these alterations cannot be established from the present measurements alone.

CONCLUSION

The study demonstrated that ingestion of greywater derived microparticles altered immune related parameters, digestive enzyme activity, nutrient composition, and some aspects of meat composition in *Gallus gallus domesticus*, with several changes being more pronounced at the highest greywater microparticles concentration (G3). These findings indicate that dietary exposure to greywater microparticles can alter physiological and nutritional parameters in chickens under the conditions investigated. However, the present study did not directly investigate the mechanisms responsible for these changes, and the effects cannot be attributed to a single constituent of the heterogeneous greywater derived particulate material. Further research is required to clarify the underlying mechanisms and to evaluate the effects of greywater microparticles exposure under different conditions and in other animal species.

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