Poultry Science

Serum metabolome responses induced by long-term inoculation of suspended PM2.5 in chicken

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ABSTRACT The adverse effects of exposure to fine particulate matter (**PM2.5**) on body health have attracted global public attention. However, there is limited research on PM2.5 in animal houses. Numerous studies have indicated that long-term exposure to high levels of PM2.5 can cause damage to multiple systems in animals. Poultry houses are one of the primary sources of PM2.5 emissions. However, there is limited research on the effects of PM2.5 exposure on poultry organisms. This study analyzed the histopathological changes in the lung tissue of poultry under PM2.5 exposure conditions. It used the LC−MS method to analyze the alterations in the serum metabolomic profile of poultry. This study confirmed that long-term exposure to high levels of PM2.5 had significantly reduced the growth performance of poultry. Histopathological slides of the lung tissue in chickens exposed to long-term retention of PM2.5 clearly showed significant damage. Furthermore, the serum metabolome analysis revealed significant changes in the serum metabolic profile of chickens exposed to long-term PM2.5 exposure. Specifically, there were notable alterations in the Glycerophospholipid metabolism, Steroid hormone biosynthesis, and Phenylalanine, tyrosine, and tryptophan biosynthesis pathways.

Key words: PM2.5, metabolomics, poultry, serum, lung damage

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INTRODUCTION

According to reports, there are approximately 6.67 million deaths globally each year due to air pollution, with 4.14 million deaths attributed to diseases caused by delicate particulate matter (Fuller et al., 2022). Fine particulate matter (**PM2.5**), the primary air pollutant, can enter small airways and alveoli. Its characteristics include small size, complex composition, and the ability to adsorb various harmful substances on its surface (Thangavel et al., 2022). Studies have shown that the particulate matter composition or water-soluble components of PM2.5 could have varying degrees of impact on human and animal health. This injury included damage to the respiratory system (Shi et al., 2019; Zhao et al., 2020; Liu, K. et al., 2022), cardiovascular system (Xie et al., 2021; Chen et al., 2022), nervous system (Thiankhaw et al., 2022; Yuan et al., 2022), reproductive system (Zhou et al., 2019; Guan et al., 2020), and an increased risk of cancer (Hamra et al., 2014; Pun et al., 2017). However, due to the complexity of the processes involved in systemic damage, some of the mechanisms by which PM2.5 caused these injuries have yet to be fully elucidated.

Metabolomics is a global analysis of metabolite levels in an organism during a specific period, aiming to elucidate the pathological or physiological changes in the organism during that period (Alseekh et al., 2021). An increasing number of studies have demonstrated that animals exposed to PM2.5 environments experience significant changes in their metabolite levels (Wang et al., 2017; Zhang et al., 2021). Due to the variations in PM2.5 composition across different environments and the differences in the physiological states of animals at different time periods, the changes in metabolites within animal bodies under PM2.5 exposure were not entirely identical. Zhang et al. (2018) conducted a study on the nontargeted metabolome of mice using ^{1}H NMR. They found that acute exposure to PM2.5 particles or water-soluble components significantly affected the mice's amino acid, lipid, and energy metabolism. Li et al. (2020) conducted a study using LC-MS to investigate the lung-targeted metabolome in mice exposed to PM2.5 in the lungs. They found that PM2.5 significantly disrupted the mice's lung amino acid metabolism, energy metabolism, and microbial community structure in the lungs. In a study conducted by Hood et al. (2022) on the

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exposure of women undergoing infertility treatment to PM2.5. They found that short-term exposure to PM2.5 significantly affected amino acid metabolism and cellular signaling in the human body. On the other hand, long-term exposure had significant impacts on amino acid metabolism, vitamin metabolism, and inflammation signaling pathways. Studying the influence of PM2.5 on metabolite levels in biological organisms is of great significance, as it can help elucidate the mechanisms underlying disease development.

China has been facing significant threats from PM2.5 pollution. According to the "2022 World Air Quality Report" published by IQAir (https://www.iqair.cn/cn/world-most-polluted-countries), although the annual average PM2.5 concentration in China has been continuously declining over the past 5 yr, the annual average PM2.5 concentration in China in 2022 (30.6 $\mu g/m^3$) remains at a relatively high level compared to the latest safety standard set by the World Health Organization (5 $\mu g/m^3$). Intensive poultry farming systems were significant hotspots of air pollution in China (Yang et al., 2018; Wu et al., 2019). However, there is currently limited research on the effects of PM2.5 on poultry metabolism. High concentrations of PM2.5 in poultry houses reduce poultry's growth performance and cause irreversible damage to their bodies (Banhazi et al., 2008; Lai et al., 2012). Furthermore, high concentrations of PM2.5 in poultry houses also threaten the health of workers and residents in the surrounding areas (Audi et al., 2017; Shen et al., 2018). In the report, Simpson suggested that respiratory disease incidence among poultry house workers was much higher than among other agricultural workers (Simpson et al., 1998). In addition, the Rimac et al. (2010) study found that poultry workers have a higher incidence of diseases related to eye, skin, and respiratory symptoms compared to others. Understanding the impact of PM2.5 on poultry metabolomics can contribute to healthy poultry production, providing insights into the mechanisms of PM2.5-induced damage to human health.

This study collected PM2.5 in the chicken house environment for exposure experiments. The serum metabolome of the chickens was characterized using LC-MS nontargeted metabolomics analysis. This study confirmed the significant impact of PM2.5 exposure on the production performance of chickens. The study also explored the effects of PM2.5 exposure on the serum metabolites of chickens and their associated metabolic pathways.

MATERIALS AND METHODS

Collection of PM2.5 Samples

The PM2.5 samples were collected from a chicken farm belonging to Guangdong Tian Nong Food Co., Ltd. (Qingyuan, Guangdong, China). The PM2.5 particles in the air were collected using an air particulate matter sampler at the central location inside the chicken house. Each sample of PM2.5 was collected continuously for 24 h at a flow rate of 100 L/min. The collected glass fiber filter membranes are subjected to fragmentation treatment using ultrasonic crushing, followed by low-speed centrifugation. This process is repeated 3 times. Afterward, the elution fluid was filtered to obtain a suspension of PM2.5 particles. The obtained PM2.5 suspension was diluted with physiological saline to a concentration of 500 $\mu g/mL$ and used for the exposure experiment on chickens. For the control group, blank filter membranes were processed using the above mentioned steps to obtain control extraction fluid. All extraction fluids are stored in a refrigerator at 4°C.

Management of Animals and Collection of Serum Samples

The Animal Welfare and Ethics Committee of Foshan University has approved the animal experiments. Twenty-seven-day-old specific-pathogen-free chickens were divided into the control group (LC) and the long-term exposure group (LE). Each group consisted of 10 chickens. After 2 d of adaptation feeding, the initial weights were measured. The control group (LC) had an initial weight of 68.35 ± 1.61 g, while the long-term exposure group (LE) had an initial weight of 68.27 ± 1.78 g. There was no significant statistical difference in weight between the groups ($P < 0.05$). The exposure and control groups were administered with PM2.5 and control extraction fluids via intranasal drops, respectively. Each administration consisted of 100 μL, given 5 times weekly from Sunday to Thursday, for 8 wk. During the experiment, the chickens had free access to food and water. At the end of the study, the chickens in each group were weighed, and the average daily weight gain of the chickens was calculated.

$$\text{Mean Daily Weight} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Days}}$$

After the completion of the exposure experiment, wing vein blood samples were collected from each group. The collected blood samples were centrifuged, and the upper layer of serum was extracted. The obtained serum samples were stored in a refrigerator at −80°C for preservation.

Analysis of Lung Tissue Pathology Sections

After the completion of the exposure experiment, the experimental chickens were euthanized, and lung samples were collected and fixed in a 10% formaldehyde solution. The lung tissue was then dehydrated and embedded in paraffin. The tissue was made into wax sections with a thickness of 5 mm. The sections were stained with Hematoxylin and Eosin (**H&E**) and Masson's trichrome stains, respectively. Optical microscopy was used for microscopic examination.

LC-MS Metabolomics Analysis

The LC/MS system used for metabolomics analysis consists of the Waters Acquity I-Class PLUS ultra-high performance liquid chromatography coupled with the Waters Xevo G2-XS QTof high-resolution mass spectrometer. The column used was purchased from Waters and is the Acquity UPLC HSS T3 column (1.8 μm, 2.1 × 100 mm). The Waters Xevo G2-XS QTOF high-resolution mass spectrometer can collect primary and secondary mass spectrometry data in MSe mode under the control of the acquisition software (MassLynx V4.2, Waters, Shanghai, China). During each data acquisition cycle, simultaneous dual-channel data acquisition can be performed at low and high collision energy. The raw data collected using MassLynx V4.2 (MassLynx V4.2, Waters, Shanghai, China) was subjected to data processing operations such as peak extraction and peak alignment using Progenesis QI (Progenesis QI, Waters, Shanghai, China) software. Identification was performed using the online METLIN database and a custom-built library in Progenesis QI (Progenesis QI, Waters, Shanghai, China) software. The identification process involved theoretical fragment identification and a mass deviation within 100 ppm.

Data Analysis

The obtained raw peak area information from the metabolomic spectra was subjected to total peak area normalization. Subsequently, principal component analysis (**PCA**) and spearman correlation analysis were conducted to assess the repeatability of the samples within the experimental group and the quality control samples. In addition, using the KEGG, HMDB, and Lipidmaps databases, the identified compounds were searched to obtain their classification and pathway information. Based on the grouping information, the fold changes were calculated and compared. The significance of differences for each compound was determined using t-tests, resulting in *P*-values. OPLS-DA modeling was performed using the R package "ropls," and the model's reliability was validated through 200 permutation tests. Differential metabolites were selected using a combination of fold change and *P*-values from the OPLS-DA model. MetaboAnalyst (MetaboAnalyst, Montreal, Canada) was used to annotate the pathways enriched with differential metabolites.

RESULTS

The Impact of PM2.5 Exposure on Animal Growth Performance

After the adaptation period, the initial weights of specific-pathogen-free chickens in each group were recorded. The weights were then measured once weekly during the 8-wk free-feeding period. The impact of long-term exposure to PM2.5 on chicken growth performance is assessed by comparing the initial weight, final weight, and average daily weight gain of chickens in the control group and the exposure group. The differences were analyzed using ANOVA, and the results are presented in Figure 1. Under the initial conditions, the 2 groups had no significant difference. However, the average final weight in the PM2.5 long-term exposed group was significantly lower than that in the control group ($P < 0.05$). Similarly, the average daily weight gain also showed the same result ($P < 0.05$). The results indicated that long-term exposure to PM2.5 significantly reduced the weight gain of chickens. The weight data of LC and LE groups at various stages as is shown in Table 1.

Changes in Lung Tissue Pathology

The effects of long-term PM2.5 exposure on the lungs of chickens were evaluated using histopathological sections stained with H&E and Masson staining. The results are shown in Figure 2. The H&E staining shows that the control group exhibits intact dense alveolar walls. In contrast, the long-term exposure group exhibits significant rupture and damage to the alveoli with a loose structure. The Masson staining reveals that the control group exhibits dense lung interstitium without significant fibrosis, while the long-term exposure group shows loose lung interstitium with evident fibrosis. The histopathological results indicated that long-term exposure to PM2.5 caused significant damage to the lungs of chickens.

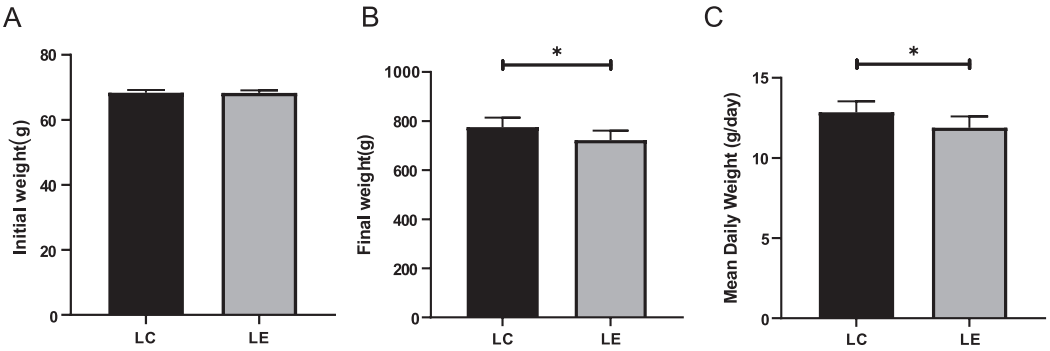

Figure 1. Weight changes in the control group and long-term exposure group of chickens. (A) Initial weight, (B) Mean final body weight, (C) Mean daily weight.

Table 1. The weight data of LC and LE groups at various stages.

Group/weight (g)	Day 9	Day 23	Day 37	Day 51	Day 65	Mean daily weight
LC1	67.19	186.69	366.15	582.71	732.22	12.09
LC2	68.67	188.37	370.80	587.21	755.54	12.49
LC3	69.66	191.57	378.15	607.13	822.80	13.69
LC4	68.24	191.25	384.62	589.11	807.03	13.41
LC5	67.52	182.98	372.70	589.95	741.61	12.26
LC6	68.86	191.20	378.82	588.57	805.10	13.39
LC7	69.17	192.92	384.95	606.36	829.56	13.81
LC8	68.35	188.36	368.70	570.99	742.81	12.26
LC9	68.80	189.72	372.53	601.38	783.65	13.00
LC10	67.01	186.69	368.76	565.92	729.56	12.05
LE1	68.24	184.47	372.65	594.43	745.07	12.31
LE2	69.04	184.7	370.18	558.65	719.96	11.83
LE3	67.36	185.88	369.53	562.13	668.28	10.93
LE4	68.25	185.3	364.46	591.63	752.23	12.44
LE5	67.57	183.21	361.61	555.81	669.36	10.94
LE6	69.59	193.86	372.84	583.74	764.16	12.63
LE7	67.77	183.51	358.52	560.84	664.69	10.85
LE8	67.34	184.98	365.23	572.45	742.31	12.27
LE9	69.18	191.69	371.77	592.82	752.67	12.43
LE10	68.31	186.07	374.42	563.65	741.46	12.24
Average/LC	68.35	188.98	374.62	588.93	774.99	12.84
Average/LE	68.27	186.37	368.12	573.62	722.02	11.89
P value	0.8276	0.0914	0.0274	0.0309	0.0073	0.0067

Discovery and Identification of Differential Metabolites

In this study, the metabolic profile in serum was detected using an LC-QTOF/MS system, and a total of 15,781 peaks were detected. Among them, 2,654 metabolites were annotated using the KEGG and HMDB databases. Differential metabolites were selected using a combination of fold change (**FC**) and *P*-values from the OPLS-DA model. Metabolites with *P*-values less than 0.05 and a log2FC greater than 0 were considered upregulated, while those with a log2FC less than 0 were considered downregulated. The cation and anion modes identified 128 and 62 differential metabolites, respectively. ROC analysis was performed to assess the specificity, sensitivity, and accuracy of differential metabolites in determining the reliability of the binary classification model. Among them, 21 differential metabolites were identified as potential biomarkers associated with long-term exposure to PM2.5 (AUC > 0.8). The information of these metabolites is presented in Table 2. Among these differential metabolites, Lipids and lipid-like molecules accounted for the highest proportion, with 16 species. This was followed by 2 species of Organoheterocyclic compounds and 2 species of Organic acids and derivatives. There was 1 species of other metabolites. At the subcategory

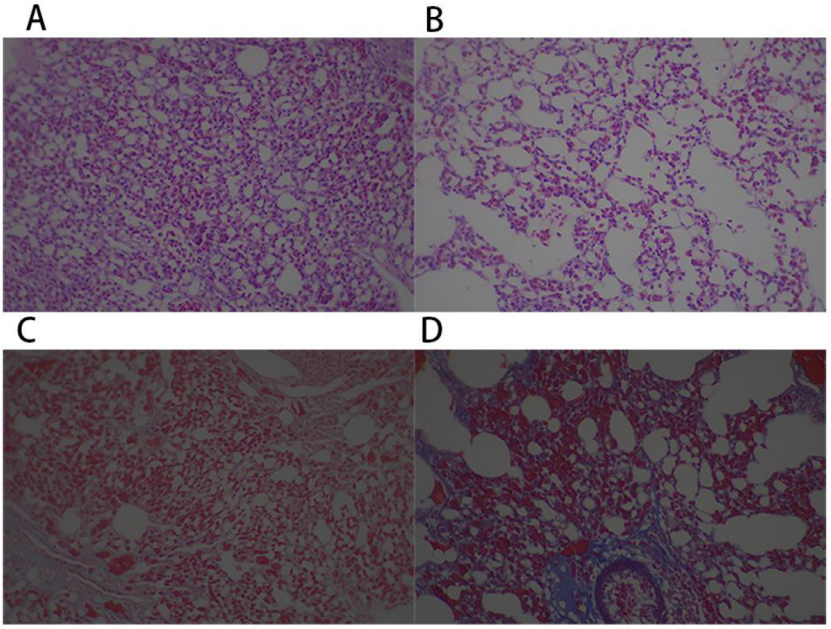

Figure 2. (A) H&E staining of lung tissue sections in the control group, (B) H&E staining of lung tissue sections in the long-term exposure group, (C) Masson staining of lung tissue sections in the control group, (D) Masson staining of lung tissue sections in the long-term exposure group.

Table 2. Distribution of different categories of differential metabolites in the serum metabolome.

Types	Subtypes	Compound names	m/z	Retention time (min)	Variation	*P* value	AUC
Lipids and lipid-like molecules	Glycerophospholipids	Glycerophosphocholine	258.11	5.73	up	0.004	1
		1-Linoleoylglycerophosphocholine	520.34	7.81	up	0.031	0.92
		PC(16:0/18:2(9Z,12Z))	758.57	9.54	up	0.006	1
		PE(16:0/0:0)	454.29	7.98	up	0.032	0.96
		LysoPC(16:1(9Z))	494.32	7.53	up	0.043	0.88
		LysoPC(18:1(11Z))	522.36	8.18	up	0.02	0.92
		LysoPE(20:3(11Z,14Z,17Z)/0:0)	538.27	8.76	down	0.003	1
	Steroids and steroid derivatives	25-hydroxyvitamin D3	439.29	7.99	up	0.038	0.92
		17alpha-Hydroxyprogesterone	353.22	3.84	down	0.042	0.88
		Androstenedione	611.35	8.76	down	0.04	0.84
		Androsterone glucuronide	508.29	8.36	down	0.034	0.88
	Endocannabinoids	2-AG	774.56	9.65	up	0.047	0.92
	Sphingolipids	Ganglioside GA2 (d18:1/16:0)	1,063.69	8.84	up	0.032	0.88
	Pteridines and derivatives	Sepiapterin	202.07	0.70	up	0.007	0.96
	Pyridines and derivatives	1-Methylnicotinamide	179.11	6.71	up	0.047	0.88
	Lactones	Mevalonolactone	95.05	0.91	up	0.022	0.88
Organic acids and derivatives	Carboxylic acids and derivatives	L-Tyrosine	182.08	1.72	up	0.016	0.92
	Organic phosphoric acids and derivatives	O-Phosphorylethanolamine	140.01	7.98	up	0.02	0.92
Organic nitrogen compounds	Organonitrogen compounds	Phosphorylcholine	184.07	8.10	up	0.012	0.96
		Choline	104.11	8.32	up	0.013	0.92
Others		4-Cholesten-7alpha,12alpha-diol-3-one	381.31	6.64	up	0.036	1

level, the differential metabolites include Glycerophospholipids, Steroids and steroid derivatives, Endocannabinoids, Sphingolipids, Pteridines and derivatives, Pyridines and derivatives, Lactones, Carboxylic acids and derivatives, and Organonitrogen compounds. This indicated that long-term exposure to PM2.5 may have impacted lipid metabolism, amino acid metabolism, and energy metabolism in the serum metabolome of chickens. The heatmap illustrated the changes in levels of differential metabolites between the control group and long-term PM2.5 exposure, using horizontal standardization, as shown in Figure 3. Under conditions of long-term PM2.5 exposure, there were 17 significantly upregulated metabolites and 4 significantly downregulated metabolites.

The Impact of Differential Metabolites on Metabolic Pathways

Based on the KEGG database, the differential impact of metabolites obtained in this study on metabolic pathways was analyzed, utilizing the MetaboAnalyst (MetaboAnalyst, Montreal, Canada) online platform.

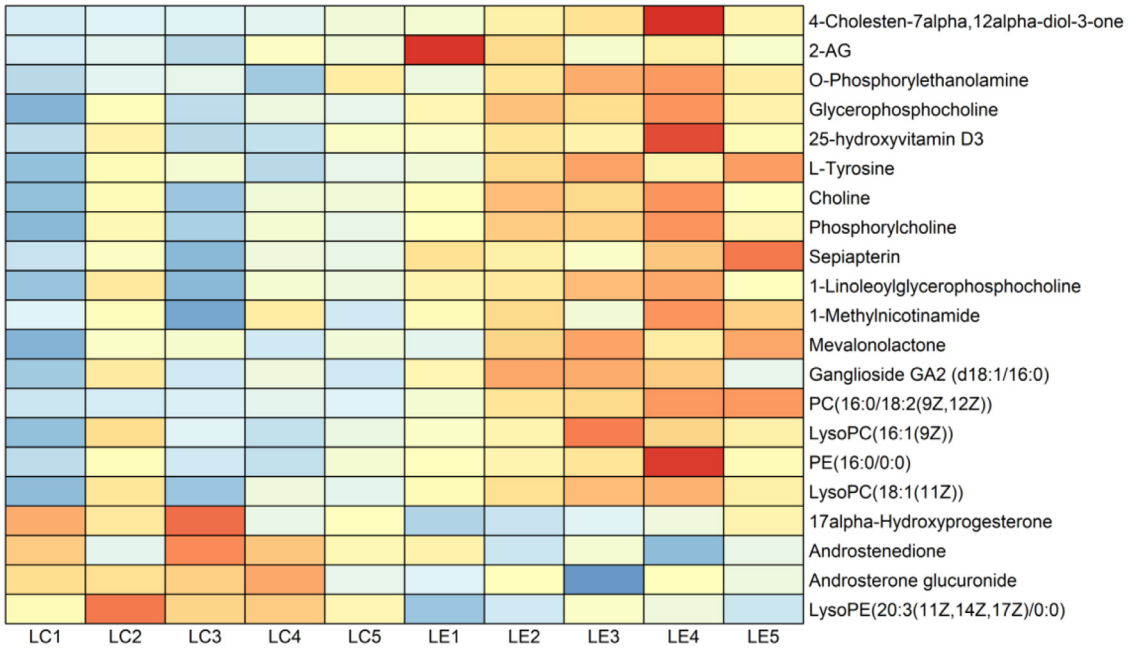

Figure 3. Heatmap showing changes in biomarkers in the control group and long-term exposure group.

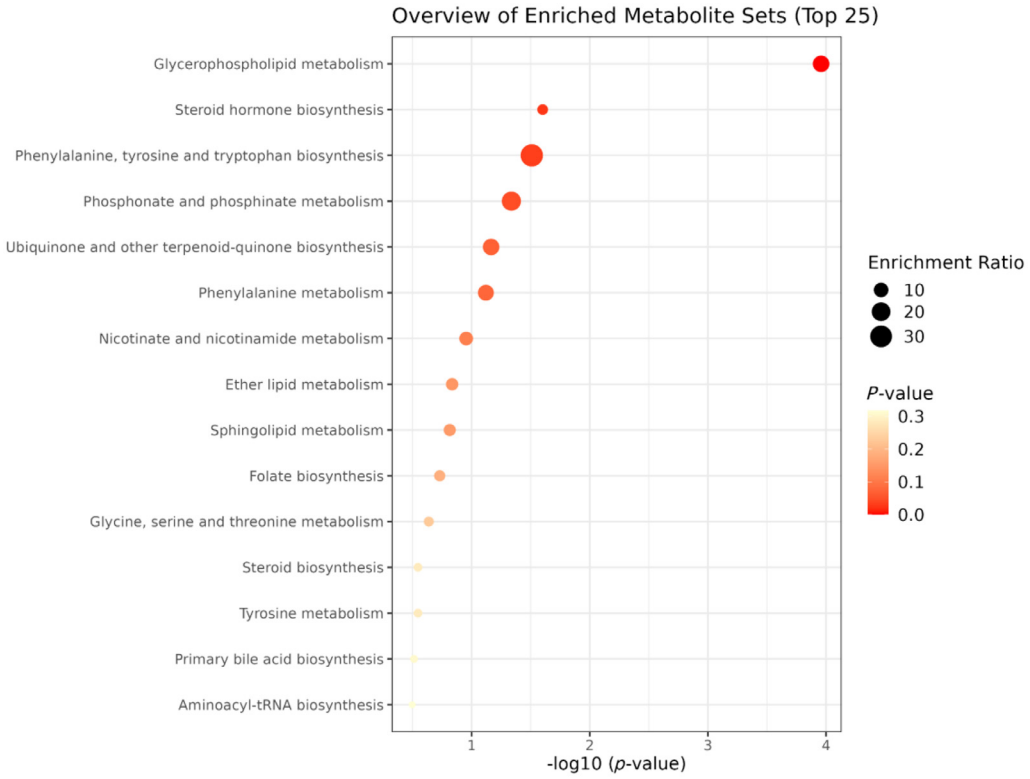

Figure 4. Bubble plot showing the enrichment of KEGG pathways.

The results are shown in Figure 4. The differential metabolites in the samples significantly perturbed the metabolic pathways of Glycerophospholipid metabolism, Steroid hormone biosynthesis, and Phenylalanine, tyrosine, and tryptophan biosynthesis ($P < 0.05$). The differential metabolites that significantly perturbed the metabolic pathways are listed in Table 3. The metabolites that significantly perturbed Glycerophospholipid metabolism include 9 species, namely Phosphorylcholine, Choline, O-Phosphoethanolamine, Glycerophosphocholine, PC(16:0/18:2(9Z,12Z)), PE(16:0/0:0), LysoPC(16:1(9Z)), LysoPC(18:1(11Z)), which showed significant upregulation (Log2FC > 0, P_value < 0.05). However, LysoPE(20:3(11Z,14Z,17Z)/0:0) exhibited significant downregulation in the serum metabolome. Following exposure to PM2.5, there was a significant decrease in the levels of 17alpha-Hydroxyprogesterone, Androstenedione, and Androsterone glucuronide in the serum. This decrease indicated a disruption in the production of steroid hormones. Additionally, the metabolite L-tyrosine exhibited upregulation, impacting the phenylalanine, tyrosine, and tryptophan biosynthesis pathway.

DISCUSSION

In this study, we conducted a comparative analysis of the effects of PM2.5 exposure on the weight variations of chickens at different developmental stages. The results revealed a significant reduction in average daily weight gain and final body weight in chickens subjected to long-term PM2.5 exposure. These findings clearly demonstrated the adverse impact of PM2.5 exposure on the growth performance of chickens. The detrimental effects of PM2.5 on both animal and human health have been extensively investigated. PM2.5 are ubiquitous in

Table 3. Distribution of differential metabolites mapped to major pathways.

Metabolic pathway	P_pathway	Compound names	P_value	Log2FC
Glycerophospholipid metabolism	1.1E-4	Phosphorylcholine	0.011549	0.487734
		Choline	0.013274	0.488752
		O-Phosphoethanolamine	0.019629	0.66311
		Glycerophosphocholine	0.004238	0.572486
		PC(16:0/18:2(9Z,12Z))	0.005721	2.049414
		PE(16:0/0:0)	0.031911	0.559487
		LysoPC(16:1(9Z))	0.042519	0.652103
		LysoPC(18:1(11Z))	0.019865	0.449854
		LysoPE(20:3(11Z,14Z,17Z)/0:0)	0.00339	−0.34189
Steroid hormone biosynthesis		17alpha-hydroxyprogesterone	0.042395	−0.19924
	0.025	Androstenedione	0.039622	−0.25539
		Androsterone glucuronide	0.034391	−0.27937
Phenylalanine, tyrosine, and tryptophan biosynthesis	0.0309	L-tyrosine	0.015787	0.015787

both natural environments and human societies. However, environments like factories or livestock farms experienced higher concentrations of PM2.5 in the past, indicating that intensive human or animal activities contributed to its generation (Ma et al., 2021). Typically, good feeding management conditions, which aim to meet animal welfare standards, have positive effects on animal growth. The observed decline in animal growth performance can be attributed, in part, to a reduction in feed intake, which directly influenced their weight. Previous studies have indicated that PM2.5 exposure led to metabolic disruptions in carbohydrates, lipids, and amino acids, resulting in a decrease in the accumulation of substances within the animal body (Ran et al., 2021; Zhao et al., 2022). The significant changes observed in lipid and amino acid metabolism in the serum metabolome of this study further support this perspective. Moreover, PM2.5 can also exert additional negative effects on animal growth performance. These effects included a significant increase in inflammatory microorganisms within the animal's gastrointestinal tract (Wang et al., 2023), heightened energy expenditure due to stress (Eid et al., 2003; Malheiros et al., 2003), and impaired cognitive function resulting from neural damage (Allen et al., 2014). Collectively, these impacts not only reduced feed intake but also hindered feed utilization efficiency. It is worth noting that PM2.5 exposure also diminished the egg-laying efficiency of poultry, potentially linked to a decrease in the concentration of Androstenedione in the serum (Richard-Yris et al., 1983). In our study, the decrease in Androstenedione indicated that long-term exposure to PM2.5 might lead to a decline in egg production in laying hens. Furthermore, hormones such as 17α-hydroxyprogesterone and testosterone glucuronide, which were considered to have positive effects on animal reproduction, exhibited decreased levels. This decrease indicated a decline in animal reproductive capacity (Brochu and Bélanger, 1987; Merlob et al., 2012). Our study demonstrated the same results. This highlighted the multifaceted consequences of PM2.5 exposure on animal health and productivity.

Meanwhile, in this study, the damage to the lungs of chickens caused by PM2.5 was confirmed. From a physical standpoint, due to its smaller particle size, PM2.5 can rapidly enter the alveoli through the respiratory tract, causing mechanical damage and subsequent disruption of the alveoli, leading to chronic inflammation (Thangavel et al., 2022). Additionally, certain harmful substances attached to PM2.5 can disrupt antioxidant proteins, increase reactive oxygen species levels, and induce damage to lung epithelial cells (Ornatowski et al., 2020). Both mechanical and oxidative damage contribute to chronic, specific inflammation in the lung interstitium and deposition of collagen, ultimately resulting in pulmonary fibrosis (Liang et al., 2019). Based on mapping at the serum metabolite level, the abnormalities in glycerophospholipid metabolism may be associated with alveolar rupture or impaired alveolar regeneration (Agudelo et al., 2020). Our study found that long-term exposure to PM2.5 led to significant lung fibrosis and alveolar rupture. This study observed abnormal glycerophospholipid metabolism, further substantiating the potential lung damage in chickens caused by PM2.5. Moreover, there was a significant increase in inflammatory factors observed in the bronchoalveolar lavage fluid of animals exposed to PM2.5 in previous studies. This provided additional support for the notion that PM2.5 induced inflammatory damage to the animals' lungs (Zhao et al., 2019).

In this study, 3 metabolic pathways significantly affected by long-term PM2.5 exposure were identified through KEGG pathway analysis. The results indicated that prolonged PM2.5 exposure significantly alters glycerophospholipid metabolism, steroid hormone biosynthesis, and the biosynthesis of phenylalanine, tyrosine, and tryptophan. Glycerophospholipids are major components of cell membranes and also serve as potential mediators of inflammation within the animal body (Zhu et al., 2022). Additionally, glycerophospholipids can be hydrolyzed by phospholipases, interact with G protein-coupled receptors, regulate intracellular signaling pathways, and participate in immune responses, cell proliferation and apoptosis, and inflammatory reactions (Makide et al., 2014; Pietzner et al., 2017; Zeng et al., 2017). This study identified abnormalities in the metabolism of 9 glycerophospholipid species, indicating that PM2.5 may affect chicken immune responses by damaging the membrane structure of chicken cells. Under conditions of exposure to PM2.5, animals exhibit abnormal glycerophospholipid metabolism, highlighting the crucial role of glycerophospholipid metabolism in the mechanisms of PM2.5-induced damage (Li et al., 2019; Yu et al., 2023). Steroid hormones play regulatory roles in metabolism, cell proliferation, and differentiation within the body (Amanatullah et al., 2002). Furthermore, this study observed abnormal metabolism of tyrosine. L-tyrosine is a precursor for the synthesis of catecholamines, and abnormal metabolism of L-tyrosine may lead to damage in the animal's cardiovascular system (Ekholm and Karppanen, 1989). The abnormalities observed in the aforementioned metabolic pathways reflected the impact of long-term PM2.5 exposure on overall inflammation.

The mechanisms of PM2.5-induced damage to animal organisms were complex and extended beyond the effects on the metabolic pathways mentioned above. In addition to the effects on the mentioned metabolic pathways, the detrimental effects of PM2.5 exposure on animal health could also manifested through disruptions in other metabolic processes. 4-Cholesten-7alpha,12alpha-diol-3-one affects the synthesis of bile acids by converting to Cholyl-CoA. Elevated levels of 4-Cholesten-7alpha,12alpha-diol-3-one in the serum may be associated with liver injury or liver disease (Kaska et al., 2016). 2-AG is an endocannabinoid, it participated in the body's immune response by regulating the production of reactive oxygen species and pro-inflammatory cytokines. This process may have occurred through the binding of 2-AG to CB2 as a chemoattractant for eosinophils, facilitating leukocyte adhesion or migration

(Turcotte et al., 2015; Frei et al., 2016; Rahaman and Ganguly, 2021). Excessive levels of 25-hydroxyvitamin D3 led to oxidative stress and promoted renal tubulointerstitial fibrosis by modulating macrophage phenotype. This process involved the upregulation of TNF-α and TGF-β1 levels in macrophages (Kusunoki et al., 2015). Sepiapterin is a tetrahydrobiopterin compound that promoted vascular regeneration and functional recovery (Shimazu et al., 2011; Yoshioka et al., 2015). The increased levels of sepiapterin in the serum seemed to confirm the damage to the cardiovascular system caused by PM2.5 exposure. Liu et al.'s (2022) study found that patients with liver cancer had higher levels of 1-Methylnicotinamide in their serum compared to normal individuals. This suggested that elevated levels of 1-Methylnicotinamide may be associated with liver damage. Cecatto et al.'s (2017) report proposed that Mevalonolactone inhibits the activity of aconitase (**ACO**), an enzyme involved in mitochondrial lipid peroxidation, in the mouse brain. This suggested that the metabolic abnormality of Mevalonolactone in the serum may induce oxidative stress and neuroinflammation. In a word, the impacts of the mentioned metabolic abnormalities on chickens manifest in various aspects. On one hand, they reflect the damage caused by PM2.5 to chicken organs or cells. On the other hand, PM2.5 reduces the effectiveness of chicken cell repair and immune responses.

In summary, this study has confirmed the effects of long-term PM2.5 exposure on the weight, lung damage, and serum metabolome of chickens. The results demonstrated that long-term PM2.5 exposure significantly altered the chicken serum metabolome, with 21 biomarkers showing significant perturbations in Glycerophospholipid metabolism, Phenylalanine, tyrosine and tryptophan biosynthesis, and Steroid hormone biosynthesis pathways. The findings supported the detrimental effects of long-term PM2.5 exposure on poultry health. However, further investigations are needed to elucidate the underlying mechanisms responsible for these adverse effects. At the same time, this study also has certain limitations, a larger sample population would better demonstrate the universality of the findings.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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