Supplementary information

Materials and methods

1. Observation of hematoxylin-eosin (H&E) staining in lung tissue

The lungs of all 42-day-old broilers in the experimental group were collected. After the fresh lung tissue was washed with normal saline, the same part was cut for a standard HE staining procedure. First, paraffin-embedded fixed and sliced, then dewaxing, hematoxylin staining, and hydrochloric acid ethanol differentiation rinsing clean, using eosin dye solution for staining and ethanol gradient dehydration, xylene soaking, air drying, sealing, and finally observe the staining effect under the microscope. 100x and 200x were selected to take pictures under the mirror (Lian et al. 2023).

2. Transmission electron microscope

The lungs of all 42-day-old broilers in the experimental group were collected, washed with 0.9% NaCl solution, and placed on ice. After that, 1.0 mm × 1.0 mm × 1.0 mm tissue samples were taken from the same part of the lung. Fixed with 2.5% glutaraldehyde for 3 hours and rinsed with 0.1 mol/L phosphate buffer 3 times for 10-15 minutes each time. Then 1% Osmic acid was used for secondary fixation, washed with phosphate buffer solution, and then replaced with pure ethanol and pure acetone after ethanol gradient dehydration. The acetone-epoxy resin mixture was embedded overnight, cut into thin slices of 50 nm-70 nm, and then stained with uranium acetate-lead citrate. The tissue structure was photographed by projection electron microscope.

3. Western blot

Tissue protein extraction: Weigh 0.1 g of lung tissue, and use scissors to cut small pieces into PBS with 1% concentration of protease inhibitor (PMSF). The residual blood in the tissue was removed by 4500 rpm centrifugal for 15 min, the liquid was discarded, and the tissue was put into the RIPA lysate containing 1% PMSF. The tissue was fully ground with a tissue grinder and moved to the EP tube, and the 10 min was placed on the ice. Finally, the supernatant was centrifuged at low temperature and high speed, and the tissue protein was obtained. SDS-PAGE electrophoresis and transfer printing in Tris-glycine buffer solution containing 20% methanol was performed to transfer the imprinted protein to the film containing nitrate and cellulose. After the transfer, rinse lightly with PBST, place it in 5% skim milk, and seal at 37 ° C for 2 hours. According to the manufacturer's requirements, the primary antibody and horseradish peroxidase-labeled secondary antibody were incubated (1 10000) and finally imaged by a chemiluminescence analyzer. β-actin was used as the internal reference protein. The details of the antibodies used are shown in Table S1 below.

Table S1 Antibodies used in the present study

Antibody name	Dilution ratio	kDa	Resource
β-actin	1: 2000	42	Abclonal Technology, China
p-IκBα	1: 350	35	WanLei Biotechnology, China
IκBα	1: 350	35	WanLei Biotechnology, China
NF-κB	1: 500	65	WanLei Biotechnology, China
IL-1β	1: 350	35	WanLei Biotechnology, China
IL-18	1: 350	22	WanLei Biotechnology, China
NLRP3	1: 500	106	WanLei Biotechnology, China
Caspase-1	1: 350	45	WanLei Biotechnology, China
ASC	1: 350	22	WanLei Biotechnology, China

GSDMD	1: 1000	53	Boster Biotechnology, China
iNOS	1: 500	130	WanLei Biotechnology, China
TNF-α	1: 1000	26	Proteintech Group, USA
COX-2	1: 1000	68	Boster Biotechnology, China
GRP78	1:500	78	WanLei Biotechnology, China
IRE1	1: 500	110	WanLei Biotechnology, China
PERK	1: 500	125	WanLei Biotechnology, China
p-PERK	1: 500	119	WanLei Biotechnology, China
eIF2α	1: 500	65	WanLei Biotechnology, China
p-eIF2α	1: 500	38	Bioss Biotechnology, China
ATF4	1: 1000	39	WanLei Biotechnology, China
ATF6	1: 500	75	WanLei Biotechnology, China
CHOP	1:350	30	WanLei Biotechnology, China
Hsp 60	1:1000	60	WanLei Biotechnology, China
Hsp70	1:1000	70	WanLei Biotechnology, China
Hsp90	1:1000	90	WanLei Biotechnology, China
H3	1:300	15	WanLei Biotechnology, China

4. RNA extraction, reverse transcription, and real-time fluorescence quantitative PCR (qRT-PCR)

The lungs of each group of chickens were taken. According to the manufacturer's instructions, use the RNA extraction kit TriZol (Invitrogen, USA) to extract the total RNA. The purified and intact RNA was reverse transcribed into cDNA by TakaraPrimeScript TMRNA reverse transcription kit. The real-time fluorescence quantitative PCR amplification system was added in the order of the instructions. The total amplification system was 10 μL, and the reaction conditions were as follows: 95 °C 30 s pre-denaturation, 95 °C 5 s denaturations, 60 °C 30 s annealing extension 40-45 cycles. All samples repeated the experiment at least 3 times. The sequence of qRT-PCR primers used is shown in Table S2 below.

Table S2 Primers used in the present study

Gene	Serial number	Forward (5′–3′)	Reverse (5′–3′)
NLRP3	XM_046918111.1	GCTCCTTGCGTGCTCTAAGACC	TTGTGCTTCCAGATGCCGTCAG
Caspase-1	XM_040687588.2	GTGCTGCCGTGGAGACAACATAG	AGGAGACAGTATCAGGCGTGGAAG
IL-1β	XM_046931582.1	GAGGAGGTTTTTGAGCCCGT	GCACGAAGCACTTCTGGTTG
IL-18	XM_015297948.4	CTGAAGGTGCGGTGGTTTTG	TCTCGAAGCGACTGAAACAA
NF-κB	NM_001396038.1	GCAGATAGCCAAGTTCAGGAT	CATTTGCTTCCCTGCATTCT
IκBα	NM_001001472.3	GGAGTAGCCCTGGTAGGTCA	CCTGCGTAGGTATTGCAGCTT
TNF-α	MF000729.1	CTTCCTGCTGGGGTGCATAG	AAGAACCAACGTGGGCATTG
COX-2	MN013407.1	TGTCCTTTCACTGCTTTCCAT	TTCCATTGCTGTGTTTGAGGT
iNOS	NM_204961.2	CCTGGAGGTCCTGGAAGAGT	CCTGGGTTTCAGAAGTGGC
IL-6	NM_204628.2	AAGTTCACCGTGTGCGAGAA	TCAGGCATTTCTCCTCGTCG
IL-8	NM204524.2	GCCTGCAGAAGAAGCCTCG	GACGGGCTCAAAAACCTCCT
CHOP	KP064314.1	GCATCCAGAAGGAAGAGCGT	GGGGACGTTGAGACAGCAAT

eIF2α	NM001006477.2	GTCCTCGCAATGCTGCCTAC	CATCCCATACTTCCATCTGTCC
GRP78	NM205491.2	GAATCGGCTAACACCAGAGGA	CGCATAGCTCTCCAGCTCATT
IRE1	NM001285499.2	CTACAGGTCGCTCCTCACATC	ATCAGTCCTTCTGCTCCCATCT
PERK	XM040700435.1	CATCACTTCCAGCACAAACG	CGGTGTTTAGGAAAGCCACT
ATF6	XM422208.7	CGTCGTCTGAACCACTTACTGA	CCTTCTTTCCTAACAGCCACAC
XBP1	NM_001006192.2	GTGCGAGTCTACGGATGTGA	CTGCTGCAGAGGAACACGTA
Hsp60	NM_001012916.3	ATGCAGGTGTTGAAGGGTCG	GAGCCGTTCTCACAACCTTTG
Hsp70	NM_001006685.1	TCCTGATGAGGCTGTTGCTT	GTCTGGTTTTGTTTGGTGGG
Hsp90	NM_001109785.2	ATCAAACTTGGCCTGGGCAT	ATCCACCTCCTCCATACGTGA
IL-17	NM_204460.2	CTCACTGGCTCCATGGGATT	GTTTGATGGGCACGGAGTTG
β-actin	NM_205518.2	CCGCTCTATGAAGGCTACGC	CTCTCGGCTGTGGTGGTGAA

5. Gene-protein interaction analysis

Using the interacting gene search tool (meta scope) and protein search tool (string) and predicting the function and interaction of differentially expressed genes and proteins. Construct a gene-gene, protein-protein interaction network.

6. Statistical analysis of data

All data were expressed as mean ± SD in at least three independent experiments. Use Spass 20 to carry out statistical analysis. T-test compared the experimental and control groups, compared the significant differences between multiple groups, and selected a single-factor analysis of variance, where appropriate. $P<0.05$ was considered to be statistically significant. * indicated that there are significant differences between groups. ** indicated that there was a very significant difference between the two groups, that is, $P<0.01$ ($0.01<P<0.05$). M-MPs and H-MPs, compared with L-MPs group #$P<0.05$, ##$P<0.01$.

Abbreviations

MPs	Microplastics	PS-MPs	Polystyrene microplastics
TNF- α	Tumor necrosis factor-α	IL-1β	Interleukin-1β
IL-6	Interleukin-6	IL-8	Interleukin-8
PERK	Protein kinase-R-like ER kinases	eIF2α	Eukaryotic translation initiation factor 2 subunit alpha
ATF4	Activating transcription factor 4	ATF6	Activating transcription factor 6
CHOP	C/EBP Homologous Protein	Caspase1	Cysteinyl aspartate-specific proteinase 1
NF-κB	Nuclear factor factor-κB	NLRP3-	Recombinant NLR family, pyrin domain-containing protein 3
GSDMD	Gasdermin D	HE	Hematoxylin-eosin
RIPA	Radio-Immunoprecipitation Assay	IL-1β	Interleukin-1β
IRE1	Ire1 Inositol-requiring enzyme-1	H3	Histone H3
p-PERK	Phospho-Protein kinase-R-like ER kinases	GRP78	Glucose-regulated protein 78

p-eIF2α	Phospho-eukaryotic translation initiation factor 2 subunit alpha	CHOP	C/EBP Homologous Protein
COX-2	Cyclooxygenase-2	qRT-PCR	Real-time fluorescence quantitative PCR
PBST	Phosphate buffer containing Tween 20	iNOS	inducible nitric oxide synthase
Hsp70	Heat shock protein 70	IκBα	Nuclear factor-κB alpha
Hsp 60	Heat shock protein 60	p-IκBα	Phospho-Nuclear factor-κB alpha
ASC	Apoptosis-associated speck-like protein containing	Hsp90	Heat shock protein 90
ER	Endoplasmic reticulum	IL-18	Interleukin-18
XBP1	X-box binding protein 1		

References

Lian, C. Y., S. Wei, Z. F. Li, S. H. Zhang, Z. Y. Wang, and L. Wang. 2023. 'Glyphosate-induced autophagy inhibition results in hepatic steatosis via mediating epigenetic reprogramming of PPARα in roosters', *Environ Pollut*, 324: 121394.