



Full-Length Article

Zinc improves meat quality by modulating lipid metabolism in necrotic enteritis challenged broilers

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ABSTRACT

The objective of this study was to investigate the effect of dietary zinc from different sources on meat quality and lipid metabolism in broilers challenged with necrotic enteritis (NE). 432 one day-old commercial Arbor Acres (AA) male broilers were randomly divided into six treatments with six replicates of 12 chicks each according to a 3 × 2 design of dietary zinc (basal diet, 60 mg/kg zinc glycinate and 60 mg/kg zinc sulfate) × NE challenge (control or NE). The results showed that a negative effect of NE challenge on growth performance as reflected in higher feed conversion ratios (FCR) and lower survival rate. Zinc glycinate increased leg muscle proportion, and also improved carcass proportions under NE challenge. Both sources of zinc had an improved effect on meat quality under NE challenge, with zinc sulfate significantly reducing pectoral muscle cooking loss and zinc glycinate increasing pectoral muscle shear force. Both sources of zinc significantly down-regulated the yellowness value b* of the pectoral muscle compared to the zinc-free treatment. The NE challenge increased the pH of pectoral muscle 45 min and 24 h after slaughter. The NE challenge resulted in an upregulation of thiobarbituric acid reactive substances (TBARS) of pectoral muscle after 24 hours storage, and both sources of zinc significantly decreased TBARS after five days storage. The non-targeted metabolomic result showed that zinc glycinate affected fatty acid biosynthesis pathways, and the NE challenge also up-regulated a fatty acid synthesis pathway, down-regulated a fatty acid oxidation pathway and significantly affected unsaturated fatty acid biosynthesis pathways. These results confirm the negative effects of the NE challenge on meat quality in broilers, with NE exacerbating lipid peroxidation and affecting fatty acid composition in the pectoral muscle. Moreover, zinc glycinate raised the proportion of dihomo- α -linolenic acid in the pectoral muscle. We conclude that both the NE challenge and zinc supplementation affect meat quality by influencing lipid metabolism in broilers to modulate lipid peroxidation and fatty acid composition.

Introduction

Clostridium perfringens-induced necrotic enteritis (NE) is a common intestinal disease in broilers and infection with one or more species of coccidia is often the causative agent and exacerbates the clinical features of NE (Moore 2024). The pathogenic mechanism of *Clostridium perfringens* is that the pathogen proliferates in the gut and releases a variety of exotoxins (Timbermont et al. 2011). Coccidia infections disrupt the

integrity of the intestinal mucosa and the intestinal microbiota balance, leading to increased intestinal permeability along with toxins entering the bloodstream (Daneshmand et al. 2022, Fathima et al., 2022). The ban on antibiotics in feed has caused resistance issues that have limited the use of anti-coccidia drugs, leading to a steady increase in NE incidences worldwide, and resulting in huge economic losses (Broom 2017).

Chicken meat is one of the most consumed meat products in the

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio; FITC-D, fluorescein isothiocyanate dextran; MDA, malondialdehyde; NE, necrotic enteritis; Zn-Gly, zinc glycinate; ZnT, zinc transporter protein.

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world (Maharjan et al. 2021). Highly heritable rapid growth genetic traits and the intensive industrial broiler production system have brought an increase in chicken meat production, but this has also led to a decline in meat quality and carcass characteristics (Nasr et al. 2021). Stocking densities, farming styles, and nutritional factors have an impact on meat quality and carcass characteristics (Marchewka et al. 2023). Benefiting from the key role minerals play in an animal’s metabolic processes, zinc is added to the feed for the promotion of animal growth performance and health status (Philippi et al. 2023). Zinc can affect fatty acid synthesis and lipid metabolism by directly regulating the activity of enzymes involved in fatty acid metabolism such as fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC) and stearyl coenzyme A desaturase-1 (SCD-1) (He et al. 2023). Zinc deficiency indirectly affects muscle growth by hindering the nucleic acid synthesis process involved in amino acid metabolism (Bogdanis et al. 2023). Zinc has been reported to improve meat quality by reducing drip loss and improving lipid oxidation in broilers and reducing the proportion of woody, PSE meat (De Grande et al. 2022). Amino acid chelated zinc is considered a more bioavailable source of zinc than inorganic zinc sources due to its resistance to phytic acid in the digestive tract and better performance in improving meat quality (Stiles et al. 2024).

The liver is the major organ for lipid synthesis and metabolism in birds, and nutritional and disease factors may lead to disorders of lipid metabolism (Calik et al. 2024). The occurrence of NE disrupts the intestinal barrier and causes intestinal inflammation along with systemic acute infections in broilers, resulting in clinical disorders such as acute splenitis and abnormalities in liver function (Shah et al. 2023). It has been observed that an NE challenge influences lipid metabolic processes in broilers by regulating fatty acid synthesis and metabolic processes in the liver (Zhou et al. 2016). Zinc has been proven to mitigate the negative effects of NE by modulating the immune function and microbial composition of the intestine (Bortoluzzi et al. 2019). There are numerous studies on the effects of zinc on meat quality and the alleviation of NE by zinc, but research on the changes in meat quality characteristics of broiler chickens under NE challenge and the modulatory effects of zinc is still lacking. This study aimed to investigate the question of whether exogenous zinc intake could affect chicken meat quality by altering lipid metabolism in the NE challenged state.

Materials and methods

All experimental procedures were approved by the Ethics Committee of Shandong Agricultural University (No. SDAUA-2022-50) and conducted in accordance with the Guide for Laboratory Animals of the Ministry of Science and Technology (Beijing, People’s Republic of China).

Animals and management

The experiment followed a 3 × 2 design of dietary zinc × NE challenge. A total of 432 one day-old Arbor Acres (AA) male chicks of similar body weight were randomly divided into three dietary treatments: NT group (basal diet), OT group (basal diet with 60mg/kg zinc glycinate), IT group (basal diet with 60mg/kg zinc sulfate), with 12 replicates of 12 broilers per treatment. Half number of broilers in each dietary treatment were divided into challenge group and the other half were divided into non-challenge group separately, ensuring that initial body weights of the same dietary treatments were close to each other at 21 days of age. Starting at 21 days of age, there were six replicates for each treatment group. The experimental period lasted for 40 days, and the birds were kept in cages with the size of 70 cm × 70 cm × 40 cm with feed and water provided ad libitum. Crumbled pellets were fed for the first 21 days of the experiment and pellets were fed from 22 days of age. The basal diet (Table 1) and zinc additives were in accordance with the recommendations of the “Compound Feed for Egg Laying Chickens and Broilers” (GB/T 5916-2020) in the People’s Republic of China. The zinc

Table 1
Composition and nutritional levels of the basal diet (air-dry basis).

Item	d 1-21	d 22-35
Ingredient (%)		
Corn	49.98	54.90
Soybean meal (46 %)	35.75	30.50
Corn protein flour (60 %)	3.80	3.10
Salt	0.28	0.28
Limestone	1.75	1.62
Dicalcium phosphate	1.55	1.40
Soybean oil	5.10	6.50
Vitamin premix	0.05	0.05
Mineral premix	0.20	0.20
Choline chloride (50 %)	0.10	0.10
Methionine (99 %)	0.35	0.35
Lysine (70 %)	0.80	0.75
Threonine (98.5 %)	0.29	0.25
Phytase (20000 U)	0.02	0.02
Total	100	100
Nutritional level		
Metabolizable energy	3100 (kcal/kg)	3200 (kcal/kg)
Crude protein	23.50	21.00
Lysine	1.39	1.20
Methionine+ cystine	1.02	0.92
Calcium	1.00	0.90
Total phosphorus	0.99	0.90
Available phosphorus	0.50	0.45

Provided per kilogram of compound diet: vitamin A, 12000 IU; vitamin D3, 5000 IU; vitamin E, 80 mg; vitamin K, 3.2 mg; vitamin B1, 3.2 mg; vitamin B2, 8.6 mg; nicotinic acid, 65 mg; pantothenic acid, 20 mg; vitamin B6, 4.3 mg; biotin, 0.22 mg; folic acid, 2.2 mg; vitamin B12, 0.017 mg; I, 1.50 mg; Fe, 80 mg; Mn, 120 mg; Se, 0.3 mg; Cu, 16 mg. The nutrition level was calculated.

levels of the diets were formulated according to the requirements of the different experimental treatments were examined with ICP-MS (Table S1). The measured total zinc content remained below 100 mg/kg, which complies with the maximum limit established by the European Food Safety Authority. During feed formulation, additional glycine was added to the other 4 groups to the same level as the zinc glycinate group to balance the amino acids.

Broilers were reared in an automated environmentally controlled house where the room temperature was raised to 32 °C 48 hours before the arrival of the chicks, and the house temperature was gradually lowered over the course of the experiment until it reached 24 °C at 21 days of age and then this temperature was maintained. Humidity was kept at an average of 70 % for the first three days of the trial and 55 % to 65 % thereafter. According to the AA broiler management handbook (2018), the birds were exposed to 23 hours of light and 1 hour of darkness for the first week of life, which changes to 20 h of light and 4 hours of darkness beginning on the seventh day. To ensure the validity of the experiment, no antibiotics and additional vaccines were used during the entire period except the coccidiostat vaccine used in the establishment of the NE model.

Establishment of the NE model

Referring to past study (Guaragni et al. 2020), the NE model was performed starting from day 23 of age by administering coccidiostat vaccine (containing live attenuated oocysts of *Eimeria tenella* PTMZ strain, *E. necatrix* PNHZ strain, *E. maxima* PMHY strain, and *E. acervulina* PAHY strain; Foshan Standard Bio-Tech Co., Ltd., Foshan, China) of 30x the vaccine label dose orally to each broiler in the challenge group on day 23 and by instilling 1 mL of broth culture containing *Clostridium perfringens* (1 × 10⁹ CFU/mL) daily from day 26 to day 32. The strain was purchased from China Veterinary Culture Collection Center (Beijing, China), which was isolated from the intestine of broilers clinically diagnosed with NE and identified as NetB toxin-positive strain A, No. CVCC2030. 1 mL of the strain was incubated in 250 mL of thioglycolate broth medium in an anaerobic cabinet at 37 °C for 24 h. Then 1 mL of the

culture was inoculated into 1 L of thioglycolate broth medium supplemented with 10 g/L starch and 15 g/L peptone for 24 h. The broilers were fasted for 8 hours prior to the oral supplementation. Broilers in the non-challenged groups were orally administered with PBS or broth culture to balance the stress response caused by catching and supplementation.

Growth performance

Feed consumption was recorded to count average daily feed intake (ADFI), all chickens were weighed on day 35 to calculate average daily gain (ADG), and ADFI/ADG was used to calculate feed conversion ratio (FCR). Residual feed in the troughs was weighed, and broilers were weighed after an eight hour fasting period. All feed and broiler weights are measured in replicate. Dead chickens were weighed and recorded to adjust FCR calculation.

Sample collection

Sample collection was performed at 40 days of age and two chickens per replicate were randomly selected for sample collection. Blood samples were collected from the wing vein using a sterile syringe from two randomly selected chickens in each replicate after 8 h of fasting. The blood samples were placed in 5 mL tubes without anticoagulant for 30 min, centrifuged at 3000 rpm for 10 min at 4 °C, and serum was separated and stored in a -20 °C freezer. After blood collection, chickens were euthanized by cervical dislocation for sample collection: pectoral muscle and liver samples were collected and stored at -80 °C for molecular and biochemical indexes.

Carcass characteristic

At 40 days of age, broilers were weighed and slaughtered. Birds were soaked in hot water at 60 °C for 60 s to facilitate feather stripping. The weight of liver, heart, spleen and bursa were weighed and recorded, abdominal fat, pectoral and leg muscles from the same side were weighed after being removed completely. Ratios or indexes were expressed as percentage of the body weight before slaughter.

Meat quality

Pectoral muscle samples, kept at 4 °C for 24 h, were used for meat quality analysis. The pH was measured at three similar locations on each sample using a meat pH meter (Star, Matthaus, Germany), and the mean value was recorded as the final pH. pH values were measured and recorded at 45 minutes (pH45min) and 24 hours (pH24h) post-slaughter. Meat color measurements were performed at three positions on the same side with a colorimeter (CR-10 PLUS, Konica Minota, Japan), and the results were expressed as luminance (L^*), redness (a^*), and yellowness (b^*) values. The hue angle (Hue) was calculated according to $\arctan(b^*/a^*)$ and expressed as h° , the color saturation Chroma (C^*) = $((a^*)^2 + (b^*)^2)^{0.5}$, and the value was taken as the average of the measurements. Approximately 5 g of the sample was weighed and hung in an air-filled plastic bag at 4 °C for 24 h. The meat was weighed to measure the drip loss value after drying the surface of meat with filter paper. 5-10 g meat pieces from each breast muscle were obtained and the meat samples were heated in a thermostatic water bath at 80 °C with the samples completely submerged in the water and a thermometer was inserted into the center of the meat samples, heated until the center of the meat samples reached a temperature of 70 °C and removed and placed in a refrigerator at 4 °C for 12 hours then weighed and recorded. Percentages were applied as values for the assessment of cooking losses. After the cooking loss determination, shear force was examined with reference to our previous work (Wang et al., 2024). The chicken meat samples were cut into six pieces of similar size and thickness and the shear force was obtained by measuring the shear force in a direction

perpendicular to the muscle fibers using the Texture Analyser model TA-XT2i (Stable Micro Systems, UK), the value of the shear force was assessed using the average of the six measurements. The parameters were set to a pre-test speed of 2.0 mm/s, a test speed of 1.0 mm/s, and a post-test speed of 5.0 mm/s over a distance of 23.0 mm. The peak shear force was recorded as WBSF when cutting through the core. The value of shear force was evaluated with the average of six measurements.

Lipid peroxidation in meat

Lipid peroxidation of meat was assessed by thiobarbituric acid reactive substances (TBARS) as described by (Valentini et al. 2020). Samples of 1 g meat were obtained from several randomly selected locations on pectoral muscles after 24 h of storage at 4 °C and after 5 days of storage, chopped and added to 4 mL of purified water and homogenized thoroughly, then 4 mL of 10 % trichloroacetic acid was added and mixed. After being filtered, 1 mL filtrate was mixed with 0.25 mL of the thiobarbituric acid solution in a water bath at 80 °C for 90 min. The samples were measured by a spectrophotometer at 532 nm after cooling to room temperature. The standard curve was plotted with 1,1,3,3-tetramethoxypropane (TMP) as the standard of TBARS, and the value of TBARS was calculated.

Serum and liver biochemical indicators

The serum concentrations of glucose (GLU), total cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) were analyzed with an automatic biochemistry analyzer (7020, Hitachi, Tokyo, Japan). TC and TG levels in liver tissue were measured using kits provided by the Nanjing Jiancheng Biotechnology Institute (Nanjing, China). The procedure was performed according to the manufacturer's instructions. The intra-batch coefficient of variation (CV) of the data did not exceed 5 % and the inter-batch CV did not exceed 8 %.

Metabolites profile analysis of liquid chromatography mass spectrometer (LC-MS)

Metabolites were extracted from meat samples of the treatments (NT, OT, CNT, COT) and detected by LC-MS. An accurate 0.2 g of meat sample was obtained and 1 mL of tissue extract (methanol and chloroform were mixed in a ratio of 9:1 and 25 % water was mixed thoroughly again) was added. The samples were thoroughly ground and then sonicated for 30 min, ice-water bath for 30 min, and then centrifuged at 12000 rpm for 10 min at 4 °C. The supernatant was carefully obtained and concentrated and dried. Add 0.2 mL of 50 % acetonitrile solution, filter and store in a special vial for mass spectrometry analysis. An aliquot of each of the samples to be tested was mixed into a quality control (QC) sample for monitoring the instrument and the confidence of the data.

Chromatography was carried out with an ACQUITY UPLC® HSS T3 (Waters, Milford, MA, USA) using a Vanquish UHPLC System (Thermo Fisher Scientific, USA) for LC analysis. Specific parameters were 2.1 mm × 100 mm × 1.8 μm. 2 μL of solution was injected from the detection vial into the sampler at 40 °C, maintaining a flow rate of 0.3 mL/min. The mobile phases for the LC-ESI (+)-MS analysis consisted of 0.1 % formic acid acetonitrile (B2) and 0.1 % formic acid (A2). Separations were performed at the following gradients: 0-1 min, 8 % B2; 1-8 min, 8 %-98 % B2; 8-10 min, 98 % B2; 10-10.1 min, 98 %-8 % B2; 10.1-12 min, 8 % B2. LC-ESI (-)-MS analysis was performed using acetonitrile (B3) and 5 mM ammonium formate (A3). The separation gradient was as follows: 0-1 min, 8 % B3; 1-8 min, 8 %-98 % B3; 8-10 min, 98 % B3; 8-10 min, 98 % B3; 10-10.1 min, 98 %-8 % B3; 10.1-12 min, 8 % B3.

Mass spectrometric detection of metabolites was performed by Orbitrap Exploris 120 (Thermo Fisher Scientific, USA). An ESI ion source was used, with positive and negative ion modes for data acquisition. The

conditions of the ion source were set as follows: sheath gas pressure (40 arb), aux gas flow (10 arb), spray voltage at 3.50 kV and -2.50 kV for ESI (+) and ESI (-), respectively, capillary temperature (325 °C), MS1 range (m/z 100-1000), MS1 resolving mode (m/z 100-1000), and MS1 resolving mode (m/z 100-1000. z 100-1000), MS1 resolving power (60000 FWHM), 4 times of data dependant scans per cycle, MS/MS resolving power (15000 FWHM), normalized collision energy (30 %), dynamic exclusion time (automatic).

Fatty acid profile detection with gas chromatography mass spectroscopy (GC-MS)

The composition of fatty acids in chicken meat from all treatments was determined by GC-MS and analyzed by fatty acid detection with a triple quadrupole gas chromatograph. A mixed standard solution of thirty-seven fatty acid methyl esters (CRM47885, Sigma-Aldrich, USA) was chosen for the calibration curve to determine the fatty acid species in the samples. The sample was grinded by liquid nitrogen in a cryomill, 1g (accurate to 0.001) of the dehydrated samples was weighed in a stoppered tube, and 0.7 mL of potassium hydroxide solution (10 mol/L) and 5.3 mL of chromatographic methanol were added, and then the sample was heated at 55 °C for 90 min, and the samples were shaken and mixed every 20 min. After the heating process was completed and cooled to room temperature, 600 µL of concentrated sulfuric acid (12 mol/L) was added and mixed, followed by heating at 55 °C for 90 min, and the sample was also shaken and mixed every 20 min. After cooling, 3 mL of n-hexane and 1 mL of saturated sodium chloride solution were added to the reaction solution for extraction and rinsing, vortexed for 5 min to mix thoroughly, and then centrifuged at 2600 g for 5 min at room temperature. The supernatant was aspirated, filtered through 0.45 µm organic phase filter membrane and placed into 2 mL of sample, and then tightened the cap of the vials and stored at -20 °C until determination.

GC-MS (TQ8030, SHIMADZU, Japan) was set up according to the following conditions: Agilent HP-innowax capillary column (30 mm × 0.25 mm × 0.25 mm); high purity helium (carrier gas) column flow rate of 1 mL/min; temperature of the inlet port of the sample port of 260 °C; injection volume of 1 µL; and set the shunt ratio of 25: 1. Temperature rising procedure: initial temperature 50 °C, hold for 1min, increase temperature to 100 °C at 25 °C / min, hold for 2 min, then increase temperature to 250 °C at 4 °C / min, hold for 10 min.

Fatty acid synthesis and metabolic gene expression in the liver

Total RNA from the liver was extracted with Trizol reagent (Invitrogen, San Diego, USA). The concentration and purity of each RNA sample was measured using a NanoDrop spectrophotometer (ND-2000, Thermo Scientific, Wilmington, USA). 1 % agarose gel electrophoresis was used to detect RNA integrity. Reverse transcription of 1 µg of total RNA was performed using PrimeScript® RT reagent kit (RR047A, TaKaRa, Japan). RT-PCR was performed using TB Green PreMix Ex Taq (RR820A, TaKaRa, Japan) in an ABI 7500 Real-Time PCR Detection System (Life Technologies) to determine relative gene expression.

The reaction procedure consisted of pre-denaturation at 95 °C for 10 s, denaturation at 95 °C for 5 s for a total of 40 cycles, and finally an annealing extension at 60 °C for 34 s. Primer sequences are demonstrated in Table S2. *β-actin* and glyceraldehyde-3-phosphate dehydrogenase (*GADPH*) were used as housekeeping genes based on their stable expression patterns validated in our previous study (Kong et al., 2023). The mean value of the housekeeping genes was used to normalize the expression of the target genes. The relative expression levels of each target gene were analyzed by the 2^{-ΔΔCt} method.

Data Analysis

All experimental data except for metabolomics profiles were analyzed as follows: Values are presented as mean ± total standard error

of the mean (SEM). Data were analyzed in replicates (n = 6) using the Shapiro-Wilk test (95 % confidence interval) performing by the software SPSS 22.0 (SPSS, Inc., Chicago, USA) followed by a two-way ANOVA with general linear model, and Tukey's post-hoc test was applied to determine differences between groups. Statistical significance was set at *P* < 0.05.

For the metabolomics data, the original mass spectrometry downlink files were converted to mz-XML file format by the MS Convert tool in Proteowizard package (v3.0.8789) (Rasmussen et al., 2022). The XCMS (v3.12.0) software package was used for peak detection, peak filtering and peak alignment to obtain the quantitative list of substances. Support vectors based on the QC samples were employed for regression correction to eliminate systematic errors. Substances with CV less than 30 % in the QC samples were then retained for subsequent analysis. The R package Ropls was adopted to perform principal component analysis (PCA) on the sample data, and the model was checked for overfitting with the replacement method of the examination. Accurate mass spectra from the public database Human Metabolome Database and mass spectrometry comparison were used for identification, and metabolites were analyzed for function and pathway with the MetaboAnalyst software package (Xia and Wishart, 2011).

Results

Growth performance

The effect of dietary zinc on growth performance of broilers under NE challenge is demonstrated in Table 2. The main effect showed that the FCR of birds was significantly increased by NE challenge (*P* = 0.049) and survival rate was significantly down-regulated (*P* = 0.016). Challenge did not affect ADFI and ADG (*P* > 0.05), while zinc also did not

Table 2
Effect of two dietary zinc source on growth performance of necrotic enteritis challenged broilers at 35 days.

Item	ADFI, g/day	ADG, g/day	FCR, g/g	Survival rate, %
NT	101.88	69.49	1.47	95.01
OT	101.05	71.81	1.41	96.67
IT	105.31	72.17	1.46	100.00
CNT	100.29	67.98	1.48	91.67
COT	103.91	69.47	1.50	95.00
CIT	103.64	70.10	1.48	95.00
Pooled SEM	4.97	4.36	0.06	5.68
Diet				
Negative	101.08	68.73	1.47	93.34
Gly-Zn	102.48	70.64	1.45	95.84
ZnSO ₄	104.47	71.13	1.48	97.50
NE Challenge				
No Challenge	102.74	71.15	1.45 ^b	97.22 ^a
Challenge	102.61	69.18	1.49 ^a	93.89 ^b
<i>P</i> value				
Diet	0.344	0.466	0.745	0.264
NE Challenge	0.945	0.244	0.049	0.016
Interaction	0.531	0.978	0.246	0.802

Values are expressed as the mean and pooled SEM (n = 6). Means in a row with no common superscript are significantly different (*P* < 0.05). Lowercase letters indicate significantly different interactions and challenge effects, uppercase letters indicate significantly different dietary effects. SEM: standard error of the mean; BW: body weight; ADFI: average daily feed intake; ADG: average daily gain; FCR: feed conversion ratio; NT: negative feed treatment, chicken fed with basal diet; OT: zinc glycinate feed treatment, chicken fed with basal diet containing 60mg/kg zinc glycinate; IT: zinc sulfate feed treatment, chicken fed with basal diet containing 60mg/kg zinc sulfate; CNT: negative feed treatment with necrotic enteritis challenge, chicken fed with basal diet and challenged with necrotic enteritis; COT: zinc glycinate feed treatment with necrotic enteritis challenge, chicken fed with basal diet containing 60mg/kg zinc glycinate and challenged with necrotic enteritis; CIT: zinc sulfate feed treatment with necrotic enteritis challenge, chicken fed with basal diet containing 60mg/kg zinc sulfate and challenged with necrotic enteritis.

cause significant differences in growth performance ($P > 0.05$).

Analysis of carcass characteristics

Table 3 presents the effect of dietary zinc on carcass characteristics and organ index of broilers under NE challenge. The dietary and challenge interaction significantly affected bursa index and carcass ratio, with zinc-free diet increasing bursa index ($P < 0.001$) and zinc glycinate increasing the carcass ratio ($P = 0.031$) under challenge. Main effects showed that zinc glycinate increased leg muscle ratio compared to the negative control group ($P = 0.017$).

Meat quality analysis

The results in Tables 4 and 5 show the effects of dietary zinc on pectoral muscle processing quality and meat color of broilers under NE challenge. The interaction of zinc and NE challenge significantly affected cooking loss and shear force, with zinc sulfate reducing cooking loss in pectoral muscle under NE challenge ($P = 0.028$), while zinc glycinate increased shear force and zinc sulfate decreased shear force in broiler meat under challenge condition ($P < 0.001$). Birds subjected to the NE challenge exhibited significantly elevated pH levels in the pectoral muscles at 45 minutes ($P < 0.001$) and 24 hours ($P = 0.036$) post-slaughter. The significant interaction of zinc and NE challenge did not affect meat color ($P > 0.05$). Both sources of zinc significantly down-regulated pectoral muscle yellowness b^* ($P = 0.029$), while NE challenge down-regulated h^o of pectoral muscle ($P = 0.045$).

The TBARS value of meat

Fig. 1 presents the effect of zinc on the level of lipid peroxidation in pectoral muscle of broilers challenged with NE through the value of TBARS. The interaction between zinc and challenge did not significantly affect the meat TBARS value ($P > 0.05$). Both sources of zinc significantly decreased TBARS after five days storage of pectoral muscle ($P = 0.015$), while NE challenge increased TBARS levels after 24 hours of storage in pectoral muscle ($P = 0.004$).

Analysis of serum and liver biochemical parameters

Tables 6 and 7 show the effect of zinc on serum biochemical parameters and liver function indexes related to lipid metabolism in broilers under NE challenge. There was no significant interaction

Table 4

Effect of two dietary zinc source on the meat quality of pectoral muscle of necrotic enteritis challenged broilers at 40 days.

Item	pH45min	pH24h	Cooking loss, %	Drip loss, %	Shear force, N
NT	6.44	6.44	14.88 ^a	3.01	28.52 ^a
OT	6.45	6.35	13.89 ^{ab}	2.70	21.04 ^c
IT	6.52	6.37	14.19 ^a	2.87	23.77 ^{bc}
CNT	6.57	6.46	14.03 ^{ab}	2.67	26.53 ^{ab}
COT	6.51	6.44	14.96 ^a	2.92	26.74 ^{ab}
CIT	6.54	6.40	12.54 ^b	2.46	22.84 ^c
Pooled SEM	0.09	0.11	1.72	0.27	4.16
Diet					
Negative	6.50	6.45	14.41 ^A	2.83	27.48 ^A
Gly-Zn	6.48	6.40	14.45 ^A	2.81	24.01 ^B
ZnSO ₄	6.53	6.38	13.33 ^B	2.67	23.44 ^B
NE					
Challenge					
No	6.46 ^b	6.38 ^b	14.30	2.86	24.44
Challenge					
Challenge	6.54 ^a	6.43 ^a	13.80	2.68	25.88
P value					
Diet	0.138	0.075	0.046	0.604	< 0.001
NE	< 0.001	0.036	0.253	0.224	0.298
Challenge					
Interaction	0.083	0.487	0.028	0.168	< 0.001

Values are expressed as the mean and pooled SEM (n = 6). Means in a row with no common superscript are significantly different ($P < 0.05$). Lowercase letters indicate significantly different interactions and challenge effects, uppercase letters indicate significantly different dietary effects. SEM: standard error of the mean. The abbreviations of the treatment groups are the same as the previous ones.

observed between zinc and NE challenge ($P > 0.05$). Both zinc glycinate and zinc sulfate reduced glucose in serum ($P = 0.013$). Compared to the other two dietary treatments, zinc glycinate significantly increased serum TG level ($P = 0.047$). NE challenge significantly raised the levels of TCHO ($P = 0.048$) and LDL ($P = 0.005$) and lowered the level of TG ($P = 0.013$) in the serum. NE challenge showed the same trend as serum for TG and TCHO levels in the liver, with TG level being decreased ($P = 0.004$) and TCHO being increased ($P = 0.004$).

LC-MS metabolite profile analysis

To verify the effect of zinc and NE challenge on meat metabolites and metabolic pathways in broilers, LC-MS metabolite profiling was

Table 3

Effect of two dietary zinc source on carcass indexes of necrotic enteritis challenged broilers at 40 days.

Items	Liver, %	Heart, %	Spleen, %	Bursa, %	Abdominal fat, %	Pectorales, %	Leg muscle, %	Carcass, %
NT	1.93	0.56	0.11	0.13 ^b	0.63	19.53	13.59	77.48 ^b
OT	1.92	0.59	0.11	0.22 ^a	0.37	18.54	14.81	77.05 ^b
IT	1.84	0.57	0.10	0.15 ^b	0.55	19.28	13.89	77.59 ^b
CNT	1.89	0.60	0.11	0.19 ^a	0.63	19.62	14.51	77.49 ^b
COT	1.81	0.55	0.09	0.19 ^a	0.35	20.19	14.81	78.82 ^a
CIT	1.89	0.59	0.10	0.12 ^b	0.75	19.08	14.58	77.67 ^b
Pooled SEM	0.12	0.09	0.01	0.02	0.06	1.69	0.96	1.32
Diet								
Negative	1.90	0.58	0.11	0.16 ^B	0.64	19.57	14.12 ^B	77.49
Gly-Zn	1.87	0.57	0.10	0.20 ^A	0.36	19.32	14.81 ^A	77.90
ZnSO ₄	1.86	0.58	0.10	0.13 ^B	0.65	19.18	14.25 ^B	77.63
NE Challenge								
No Challenge	1.89	0.57	0.11	0.16	0.52	19.12	14.17	77.36 ^b
Challenge	1.86	0.58	0.10	0.17	0.58	19.63	14.63	77.97 ^a
P value								
Diet	0.512	0.962	0.450	< 0.001	0.125	0.762	0.017	0.466
NE Challenge	0.306	0.861	0.145	0.897	0.651	0.240	0.190	0.041
Interaction	0.140	0.317	0.229	< 0.001	0.708	0.179	0.210	0.031

Values are expressed as the mean and pooled SEM (n = 6). Means in a row with no common superscript are significantly different ($P < 0.05$). Lowercase letters indicate significantly different interactions and challenge effects, uppercase letters indicate significantly different dietary effects. SEM: standard error of the mean. The abbreviations of the treatment groups are the same as the previous ones.

Table 5

Effect of two dietary zinc source on the meat colour of necrotic enteritis challenged broilers at 40 days.

Item	L*	a*	b*	C*	h°
NT	55.91	3.82	7.98	8.48	64.56
OT	54.67	4.41	7.53	8.92	62.98
IT	55.14	3.94	7.90	9.01	65.23
CNT	54.70	4.23	8.67	9.79	62.55
COT	56.42	3.95	7.70	8.70	62.95
CIT	55.63	3.85	7.24	7.98	62.58
Pooled SEM	2.09	0.65	0.84	0.76	3.05
Diet					
Negative	55.30	4.00	8.34 ^A	8.05	63.61
Gly-Zn	55.49	4.17	7.63 ^B	8.82	62.96
ZnSO ₄	55.37	3.89	7.57 ^B	8.49	63.82
NE Challenge					
No Challenge	55.25	4.06	7.80	8.81	64.32 ^a
Challenge	55.52	3.99	7.86	8.75	62.70 ^b
P value					
Diet	0.940	0.375	0.029	0.234	0.629
NE Challenge	0.533	0.773	0.809	0.946	0.045
Interaction	0.106	0.123	0.184	0.010	0.389

Values are expressed as the mean and pooled SEM (n = 6). Means in a row with no common superscript are significantly different ($P < 0.05$). Lowercase letters indicate significantly different interactions and challenge effects, uppercase letters indicate significantly different dietary effects. SEM: standard error of the mean; L* = lightness; a* = redness; b* = yellowness; C* = chroma; h° = hue. The abbreviations of the treatment groups are the same as the previous ones.

implemented. Considering the economic cost, samples from NT, OT, CNT and COT groups were examined.

Figures S1 illustrates the quality control of the samples, exhibiting consistent retention time points and ion intensities at ESI+ and ESI. PCA (principal component analysis) and RSD (relative standard deviation) are commonly performed statistical analyses in non-targeted metabolomics (Figures S2 and 3). The analysis of Figure S2A and Figure S3A with regard to the PCA analysis reveals 24.9 % of the total variation in positive ion mode and 16.1 % of the total variation in negative ion mode, showing that the samples have a tendency to cluster. Quality control analysis showed that 65 % of characteristic peaks had RSD values < 30 %, demonstrating data reliability. Figure S2B indicates that 84.8 % of

features in positive ion mode met the RSD < 30 % criterion, while Figure S3B shows 77.2 % in negative ion mode.

Twenty-four metabolites were up-regulated and eleven metabolites were down-regulated in the COT group compared to the OT group. The metabolites were identified by the HMDB database and pathway analysis was performed for up- and down-regulated metabolites. The comparison of NT and OT is displayed in Fig. 2. Fig. 2A shows that fatty acid biosynthesis metabolism has one up-regulated pathway and one down-regulated pathway, respectively. Pathway enrichment indicated significant differences in fatty-acid biosynthesis between NT and OT (Fig. 2B). According to the results in Fig. 2C and D, zinc glycinate up-regulated oleic acid content and down-regulated palmitoleic acid content in pectoral muscle compared to the NT group.

The results for the comparison of OT and COT were similar to the previous conclusions that there was one upregulated and one down-regulated pathway for fatty acid biosynthesis metabolism, and the fatty acid biosynthesis pathways of the two groups showed significant differences. Challenge downregulated the oleic acid content in the pectoral muscle and upregulated the palmitoleic acid content which is presented in Fig. 3. In addition, Fig. 3B shows that NE challenge also affected the unsaturated fatty acid biosynthesis pathway and the linoleic acid metabolism pathway.

Fatty acid profiles in meat

After confirming by non-targeted metabolomics assays that both zinc glycinate and NE challenge affected the fatty acid synthesis and metabolism, we examined the fatty acid profiles of pectoral muscle from all treatments with GC-MS and classified the fatty acids according to different fatty acid classifications. Table 8 presents the effect of zinc on saturated fatty acid (SFA) content in broiler pectoral muscle under NE challenge, and significant interactions between dietary zinc and NE challenge were not observed ($P > 0.05$). The main effect showed that NE challenge resulted in downregulation of C20:0 (arachidic acid) content in the pectoral muscle ($P = 0.002$). The results of monounsaturated and polyunsaturated fatty acids content in meat were presented in Tables 9 and 10. The interaction of zinc and challenge had no significant effect ($P > 0.05$) on the content of the monounsaturated fatty acids (MUFA) and

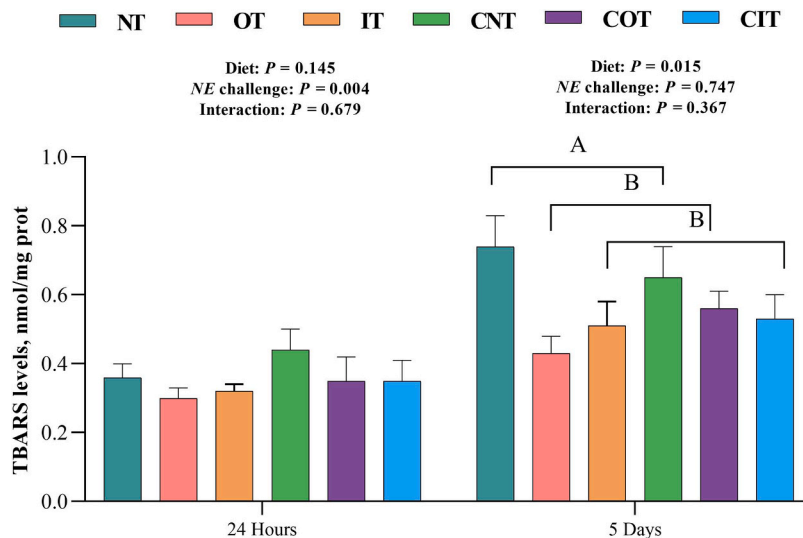


Fig. 1. Effect of dietary zinc on the TBARS levels in the pectoral muscle of necrotic enteritis challenged broilers.

Means with no common superscripts differ significantly ($P < 0.05$), with lowercase letters indicating significantly different interactions and uppercase letters indicating significantly different dietary effects. TBARS: thiobarbituric acid reactive substances; NT: negative feed treatment, chicken fed with basal diet; OT: zinc glycinate feed treatment, chicken fed with basal diet containing 60 mg/kg zinc glycinate; IT: zinc sulfate feed treatment, chicken fed with basal diet containing 60 mg/kg zinc sulfate; CNT: negative feed treatment with necrotic enteritis challenge, chicken fed with basal diet and challenged with necrotic enteritis; COT: zinc glycinate feed treatment with necrotic enteritis challenge, chicken fed with basal diet containing 60 mg/kg zinc glycinate and challenged with necrotic enteritis; CIT: zinc sulfate feed treatment with necrotic enteritis challenge, chicken fed with basal diet containing 60 mg/kg zinc sulfate and challenged with necrotic enteritis.

Table 6
Effect of two dietary zinc source on the serum biochemical parameters of necrotic enteritis challenged broilers at 40 days.

Item	GLU, mmol/L	TCHO, mmol/L	TG, mmol/L	HDL, mmol/L	LDL, mmol/L
NT	11.53	2.81	0.61	2.03	0.47
OT	10.48	2.58	0.63	2.04	0.55
IT	10.83	2.55	0.52	1.89	0.51
CNT	11.47	2.74	0.43	2.05	0.57
COT	10.83	2.83	0.55	1.97	0.86
CIT	10.01	2.93	0.47	2.13	0.61
Pooled SEM	0.92	0.30	0.10	0.25	0.11
Diet					
Negative	11.50 ^A	2.78	0.52 ^B	2.04	0.53
Gly-Zn	10.67 ^B	2.71	0.59 ^A	1.91	0.61
ZnSO ₄	10.56 ^B	2.78	0.49 ^B	2.03	0.57
NE Challenge					
No Challenge	10.95	2.65 ^b	0.58 ^a	1.92	0.51 ^b
Challenge	10.95	2.84 ^a	0.49 ^b	2.05	0.61 ^a
P value					
Diet	0.013	0.814	0.047	0.380	0.189
NE	0.560	0.048	0.013	0.118	0.005
Challenge					
Interaction	0.323	0.156	0.712	0.509	0.972

Values are expressed as the mean and pooled SEM (n = 6). Means in a row with no common superscript are significantly different ($P < 0.05$). Lowercase letters indicate significantly different interactions and challenge effects, uppercase letters indicate significantly different dietary effects. SEM: standard error of the mean; GLU = glucose; TCHO = total cholesterol; TG = triglycerides; HDL = high density lipoprotein; LDL = low density lipoprotein. The abbreviations of the treatment groups are the same as the previous ones.

Table 7
Effect of two dietary zinc source on the TG and TCHO contents of the liver of necrotic enteritis challenged broilers at 40 days.

Item	TG, nmol/g prot	TCHO, nmol/g prot
NT	0.17	0.20
OT	0.15	0.21
IT	0.15	0.20
CNT	0.11	0.22
COT	0.11	0.23
CIT	0.09	0.24
Pooled SEM	0.01	0.03
Diet		
Negative	0.14	0.21
Gly-Zn	0.13	0.22
ZnSO ₄	0.12	0.22
NE Challenge		
No Challenge	0.15 ^a	0.20 ^b
Challenge	0.10 ^b	0.23 ^a
P value		
Diet	0.532	0.656
NE Challenge	0.004	0.004
Interaction	0.820	0.369

Values are expressed as the mean and pooled SEM (n = 6). Means in a row with no common superscript are significantly different ($P < 0.05$). Lowercase letters indicate significantly different interactions and challenge effects, uppercase letters indicate significantly different dietary effects. SEM: standard error of the mean; TG = triglycerides; TCHO = total cholesterol. The abbreviations of the treatment groups are the same as the previous ones.

polyunsaturated fatty acids (PUFA). Zinc glycinate significantly increased C20:2 (eicosadienoic acid) content in meat compared to zinc-free treatment ($P = 0.037$). Zinc glycinate treatment significantly increased C20:3n3 (dihomo- α -linolenic acid) content in meat compared to zinc sulfate treatment ($P = 0.029$). Moreover, NE challenge significantly reduced C20:3n3 content in pectoral muscle ($P = 0.017$). Although dietary and challenge factors affected the content of several saturated and polyunsaturated fatty acids, they did not significantly affect the overall proportions of SFA, MUFA and PUFA in pectoral muscle (Table 11).

Lipid metabolism-related gene expression in the liver

To investigate the reasons for the regulation of fatty acid content in meat and lipid metabolism in birds, gene expression levels of fatty acid synthesis (Fig. 4A) and fatty acid oxidation (Fig. 4B) genes were examined in the liver. There was no significant interaction observed between zinc supplementation and NE challenge for expression levels of liver fatty acid synthesis genes ($P > 0.05$). As compared to the negative control, both zinc glycinate and zinc sulfate significantly decreased the gene expression level of liver ACC ($P = 0.049$). Meanwhile, NE challenge upregulated the expression level of sterol regulatory element binding protein 1c (*SREBP-1C*) ($P = 0.003$). The interaction indicated that zinc glycinate significantly decreased the expression level of liver PPAR- α in response to NE challenge ($P = 0.011$).

Discussion

Despite the benefits of intensive livestock farming with the support of modern poultry breeding technology, such as labor savings and a large amount of animal protein to the market, modern breeding techniques promoting more vigorous metabolism and the prohibition of antibiotics in feed lead to more pathogen susceptibility in broilers (Van Limbergen et al. 2020). One such pathogen is *Clostridium perfringens* and the subsequent NE that establishes after infection directly affects gut health of broilers by disrupting the intestinal barrier function, hindering nutrient absorption, and finally impacting growth performance (Goo et al. 2024). Based on our past work, NE challenge model was established following validated protocols using a standardized coccidia vaccine dose and NetB toxin-positive *Clostridium perfringens* strain CVCC2030 (Song et al., 2023). The The coccidial infection initially damages intestinal integrity, plasma leakage into the lumen, promotes the colonization of *Clostridium perfringens* and the development of clinical or sub-clinical form of NE, which finally leads to a decrease in broiler growth performance (Fathima et al. 2022). As demonstrated in our companion study, the NE challenge model increased intestinal permeability and lesion scores in broilers but did not cause substantial mortality, confirming the successful establishment of a sub-clinical NE model (Xiao 2024). In the present study, NE reduced survival rate and increased FCR in broilers, showing a negative effect of NE on growth performance.

Nutritional status and diseases could affect muscle development and consequently the carcass ratio of poultry. It was found that 30 mg/kg of organic zinc increased carcass percentage in broilers (Rossi et al. 2007). However, there is also a report revealing that different sources of zinc did not affect carcass ratio and abdominal fat percentage in broilers (Qudsieh et al. 2018). In our work, the main effect of diet showed that 60 mg/kg of zinc glycinate increased the leg muscle percentage and the interaction showed that zinc glycinate increased carcass ratio under NE challenge, suggesting that zinc promotes anabolism and improves meat production efficiency in broilers. In general, the metabolism of NE challenged broilers causes a systemic immune response due to the disruption of the intestinal barrier, consuming energy to maintain the barrier function (Emami et al., 2019). In contrast, birds increase the uptake of glucose in the gut and the translocation of glucose from the liver to ensure the energy supply (Mayorga et al. 2020). High dietary energy concentration reduces carcass yield, especially high energy paired with low protein diets leads to lower carcass percentage and increased body fat in broilers, whereas feeding diets with high protein and energy nutrient concentrations improves carcass yield (Mir et al. 2017). Modern industrial broiler production typically employs high-energy, high-protein diets to meet rapid growth demands. In the present study, the experimental diets followed this industry standard formulation to maintain real-world relevance which may have ensured enough nutrient supply during the NE challenge period. Our results revealed a significant interaction between dietary zinc and NE challenge in bursal organ index, with NE challenge increasing bursal index in

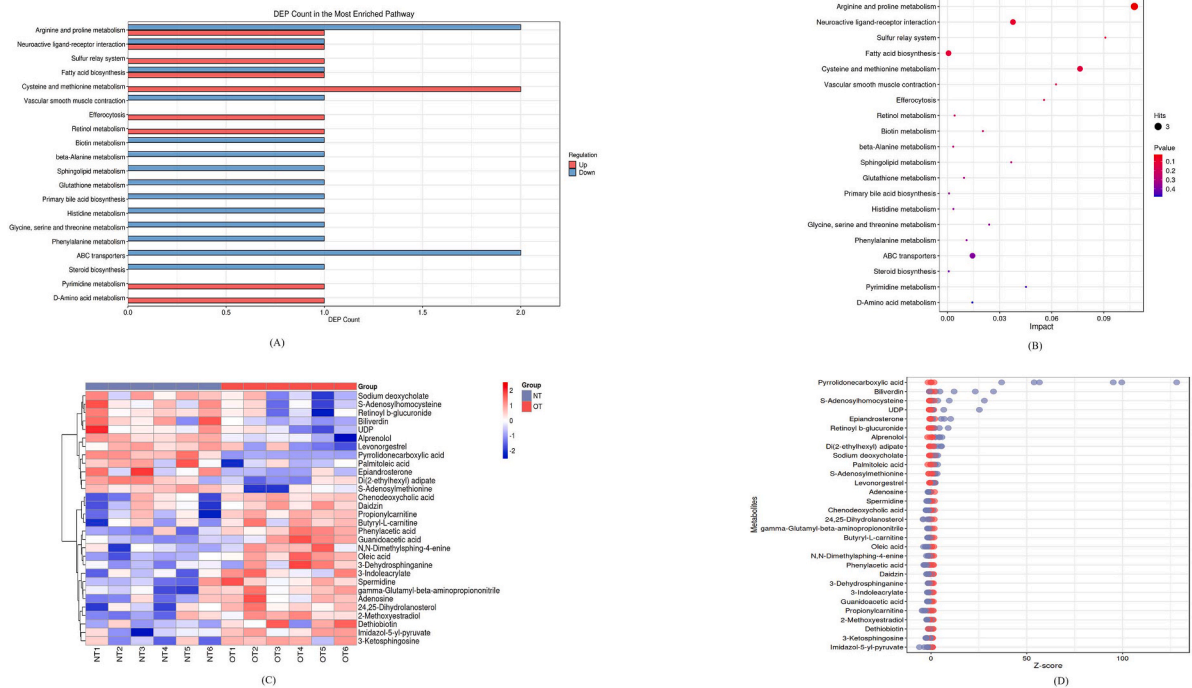


Fig. 2. Effect of dietary zinc on the number of enriched metabolic pathways (A), the bubble plots of factors influencing metabolic pathways (B), the heatmap of metabolite profiles (C) and the relative levels of differential metabolites (D) in the pectoral muscle of broilers.

A: Comparison of the number of metabolite enrichment pathways between NT and OT groups; B: Comparison of the degree of KEGG pathway enrichment in the NT and OT groups. Each bubble represents a metabolic pathway and the size of the bubble represents the impact factor; the redder the bubble colour, the more significant the metabolite enrichment; C: Comparison of metabolites between NT and OT groups; D: Comparison of the relative levels of metabolites in the NT and OT groups. The abbreviations of the treatment groups are the same as the previous ones.

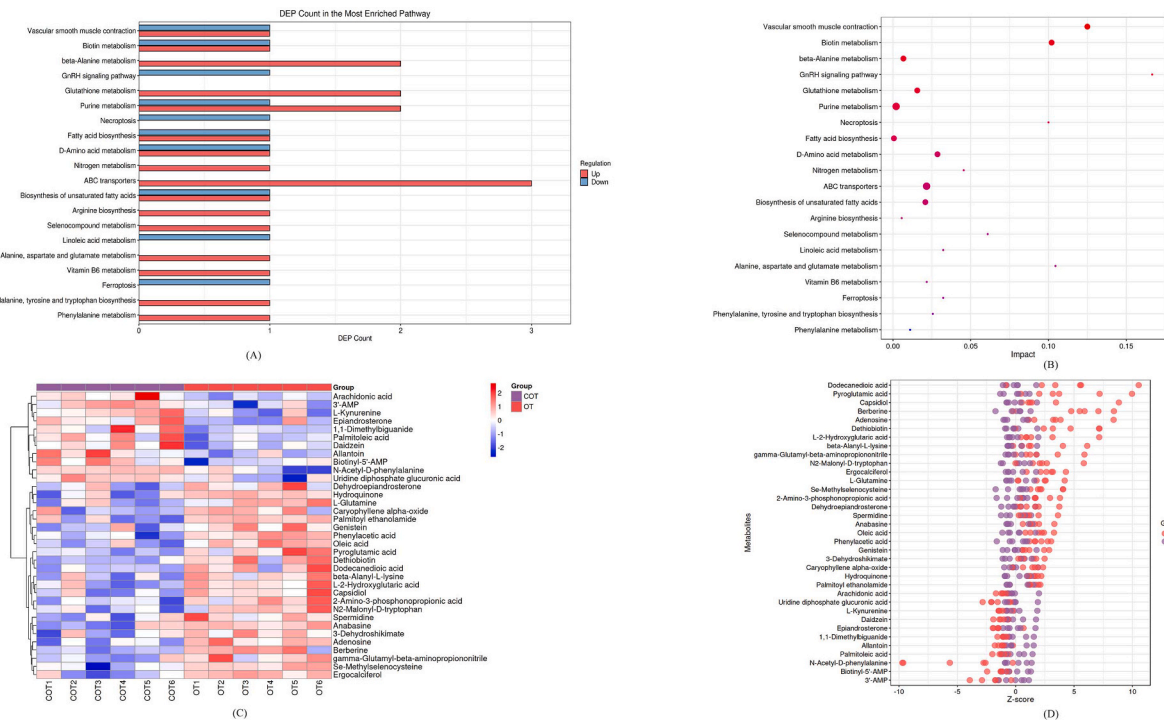


Fig. 3. Effect of necrotic enteritis challenge on the number of enriched metabolic pathways (A), the bubble plots of factors influencing metabolic pathways (B), the heatmap of metabolite profiles (C) and the relative levels of differential metabolites (D) in the pectoral muscle of broilers.

A: Comparison of the number of metabolite enrichment pathways between OT and COT groups; B: Comparison of the degree of KEGG pathway enrichment in the OT and COT groups. Each bubble represents a metabolic pathway and the size of the bubble represents the impact factor; the redder the bubble colour, the more significant the metabolite enrichment; C: Comparison of metabolites between OT and COT groups; D: Comparison of the relative levels of metabolites in the OT and COT groups. The abbreviations of the treatment groups are the same as the previous ones.

Table 8
Effect of two dietary zinc source on the saturated fatty acids profile in the meat of necrotic enteritis challenged broilers at 40 days.

Item	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0
NT	0.06	0.71	0.19	13.34	0.49	14.52	0.60
OT	0.06	0.69	0.19	13.73	0.52	15.20	0.53
IT	0.07	0.89	0.22	14.02	0.54	15.26	0.33
CNT	0.07	0.85	0.22	13.73	0.54	14.76	0.28
COT	0.06	0.80	0.20	14.01	0.51	14.97	0.24
CIT	0.06	0.73	0.19	13.69	0.53	15.21	0.25
Pooled SEM	0.01	0.14	0.02	0.43	0.05	0.54	0.02
Diet							
Negative	0.06	0.78	0.20	13.53	0.51	14.64	0.44
Gly-Zn	0.06	0.74	0.20	13.87	0.52	15.09	0.39
ZnSO ₄	0.06	0.81	0.20	13.85	0.53	15.23	0.23
NE Challenge							
No Challenge	0.06	0.76	0.20	13.69	0.52	14.99	0.49 ^a
Challenge	0.06	0.79	0.20	13.81	0.52	14.98	0.26 ^b
P value							
Diet	0.465	0.633	0.795	0.206	0.830	0.091	0.161
NE Challenge	0.799	0.613	0.720	0.507	0.763	0.964	0.002
Interaction	0.183	0.066	0.074	0.202	0.567	0.684	0.237

Values are expressed as the mean and pooled SEM (n = 6). Means in a row with no common superscript are significantly different ($P < 0.05$). Lowercase letters indicate significantly different interactions and challenge effects, uppercase letters indicate significantly different dietary effects. SEM: standard error of the mean; C12:0 = lauric acid; C14:0 = myristic acid; C15:0 = pentadecanoic acid; C16:0 = palmitic acid; C17:0 = margaric acid; C18:0 = stearic acid; C20:0 = arachidic acid. The abbreviations of the treatment groups are the same as the previous ones.

Table 9
Effect of two dietary zinc source on the MUFAs profile in the meat of necrotic enteritis challenged broilers at 40 days.

Item	C16:1n7	C17:1n7	C18:1n9c	C20:1	C22:1n9
NT	2.87	0.19	20.66	0.61	0.10
OT	2.20	0.16	21.09	0.58	0.09
IT	2.94	0.10	20.82	0.67	0.11
CNT	3.08	0.07	21.15	0.68	0.08
COT	2.88	0.08	20.57	0.58	0.08
CIT	2.60	0.11	21.19	0.56	0.08
Pooled SEM	0.72	0.01	0.50	0.09	0.01
Diet					
Negative	2.97	0.13	20.90	0.65	0.09
Gly-Zn	2.54	0.12	20.83	0.58	0.09
ZnSO ₄	2.77	0.11	21.01	0.61	0.09
NE Challenge					
No Challenge	2.67	0.15 ^a	20.86	0.62	0.10 ^a
Challenge	2.60	0.09 ^b	20.97	0.61	0.08 ^b
P value					
Diet	0.523	0.779	0.798	0.791	0.480
NE Challenge	0.560	0.030	0.204	0.422	0.008
Interaction	0.413	0.189	0.590	0.195	0.295

Values are expressed as the mean and pooled SEM (n = 6). Means in a row with no common superscript are significantly different ($P < 0.05$). Lowercase letters indicate significantly different interactions and challenge effects, uppercase letters indicate significantly different dietary effects. SEM: standard error of the mean; C16:1n7 = palmitoleic acid; C17:1n7 = margaroleic acid; C18:1n9c = oleic acid; C20:1 = gadoleic acid; C22:1n9 = erucic acid. The abbreviations of the treatment groups are the same as the previous ones.

zinc-free broilers. The bursa, as a primary lymphoid organ, plays a pivotal role in B lymphocyte development and antibody production, and zinc has the function of promoting the growth of immune organs (Molinet et al. 2023). Previous study has shown that acute inflammation induced by immune challenges in the state of zinc deficiency leads to edema of the bursal, which explains these results (Bilal et al. 2023).

Quantitative parameters of meat quality include sensory parameters of meat and processing characteristics. Sensory parameters include the color, texture, odor and flavor of the meat, while processing characteristics of meat include shear force, water holding capacity (WHC) and pH value (Barbut et al. 2022). Consumers usually make decisions based on sensory experiences when purchasing chicken, such as tenderness and color (Pellattiero et al., 2020). Excessive yellowness b* represents an undesirable color in the meat sensory, and the reduction of b* by zinc

shows the benefit of zinc on sensory characteristics in meat quality (Marchewka et al. 2023). Evidence suggests that there is a highly significant positive correlation between muscle pH and meat color due to an increased rate of conversion of glycogen to lactic acid after slaughter (Mir et al. 2017). Hydrolysis of myogenic fibers in chicken meat after slaughter leads to weakening of muscle fibers, affecting the integrity of muscle fibers, and a significant increase in the mobility and proportion of water outside the muscle fibers, resulting in increased toughness (Li et al., 2016). Previous research has shown that enhancing the antioxidant capacity of meat improves its WHC, and dietary zinc may contribute to this effect through its antioxidant properties (Skřivanová et al. 2017). Although no study has directly established the link between NE and meat quality, it has been demonstrated that immune challenge-induced oxidative stress will affect meat quality (Choi et al. 2023). Zinc significantly reduced the TBARS value of pectoral meat, suggesting that zinc reduces the level of lipid peroxidation in chicken meat by improving the oxidative status. Lipid peroxidation is the process by which reactive oxygen species are converted to non-free radical lipid breakdown species, and this process amplifies reactive oxygen species hazards through a chain reaction (Asido et al. 2024). The lipid peroxidation status of meat directly reflects the level of meat quality and affects the shelf-life of the meat. NE challenge increased the lipid peroxidation status of chicken meat after 24 hours of storage, while dietary zinc improved the lipid peroxidation status of chicken meat after 5 days of storage, confirming the negative effect of NE challenge on lipid peroxidation and the ameliorating effect of zinc on lipid peroxidation. Both zinc glycinate and zinc sulfate improved the WHC, as evidenced by reduced cooking loss of pectoral muscle and lower shear values in challenged birds. Zinc glycinate reduced the cooking loss of pectoral muscles, while zinc sulfate reduced the cooking loss of pectoral muscle under NE challenge, as well as the reduction of chicken shear by both sources of zinc suggests that zinc enhances WHC.

Research has confirmed that lipid metabolism in broilers could be regulated by nutritional methods, which in turn modulates the fatty acid composition of chicken meat and improves meat quality (Poorghasemi et al. 2013). Unsaturated fatty acids are considered beneficial to human health, especially PUFAs could help reduce the risk of cardiovascular disease (Zarate et al. 2017). Chicken meat is an important source of PUFAs, but it is prone to lipid peroxidation due to the instability of unsaturated fatty acids. Up to now, there is no published work to show the association of NE challenge and zinc with the fatty acid composition of chicken meat. The application of zinc glycinate increased the

Table 10
Effect of two dietary zinc source on the polyunsaturated fatty acids profile in the meat of necrotic enteritis challenged broilers at 40 days.

Item	C18:2n6t	C18:3n6	C18:3n3	C20:2	C20:3n6	C20:4n6	C20:3n3	C20:5n3	C22:2
NT	19.57	0.69	3.77	2.77	2.18	11.98	0.48	0.83	3.41
OT	19.49	0.81	3.74	3.15	2.34	11.50	0.49	0.78	2.67
IT	19.60	0.71	3.88	2.85	2.53	10.42	0.45	0.78	2.85
CNT	20.03	0.74	4.15	2.78	2.33	9.67	0.42	0.76	3.60
COT	19.96	0.64	3.79	2.93	2.45	10.86	0.48	0.79	3.14
CIT	19.86	0.65	3.68	2.70	2.35	11.20	0.40	0.76	3.24
Pooled SEM	0.98	0.10	0.47	0.26	0.25	1.66	0.06	0.09	0.65
Diet									
Negative	19.80	0.71	3.96	2.78 ^B	2.25	10.84	0.45 ^{AB}	0.79	3.50
Gly-Zn	19.73	0.72	3.76	3.04 ^A	2.40	11.18	0.48 ^A	0.78	2.91
ZnSO ₄	19.73	0.68	3.78	2.77 ^B	2.44	10.81	0.42 ^B	0.77	3.04
NE Challenge									
No Challenge	19.55	0.74	3.80	2.92	2.35	11.30	0.47 ^a	0.80	2.98
Challenge	19.95	0.67	3.87	2.81	2.38	10.58	0.43 ^b	0.77	3.24
P value									
Diet	0.822	0.647	0.688	0.037	0.311	0.308	0.029	0.845	0.176
NE Challenge	0.606	0.142	0.712	0.263	0.817	0.887	0.017	0.474	0.202
Interaction	0.900	0.125	0.522	0.664	0.382	0.214	0.711	0.689	0.902

Values are expressed as the mean and pooled SEM (n = 6). Means in a row with no common superscript are significantly different ($P < 0.05$). Lowercase letters indicate significantly different interactions and challenge effects, uppercase letters indicate significantly different dietary effects. SEM: standard error of the mean; C18:2n6t = linoleic acid; C18:3n6 = gamma-linoleic acid; C18:3n3 = alpha-linoleic acid; C20:2 = eicosadienoic acid; C20:3n6 = dihomo- γ -linolenic acid; C20:4n6 = arachidonic acid; C20:3n3 = dihomo- α -linolenic acid; C20:5n3 = eicosadienoic acid; C22:2 = docosadienoic acid. The abbreviations of the treatment groups are the same as the previous ones.

Table 11
Effect of two dietary zinc source on the classification of fatty acids profile in the meat of necrotic enteritis challenged broilers at 40 days.

Item	Total SFA	Total MUFA	Total PUFA
NT	29.90	24.41	45.69
OT	30.92	24.12	44.97
IT	31.31	24.63	44.06
CNT	30.43	25.06	44.52
COT	30.79	24.19	45.03
CIT	30.65	24.54	44.82
Pooled SEM	0.68	0.96	1.25
Diet			
Negative	30.16	24.74	45.11
Gly-Zn	30.85	24.16	45.00
ZnSO ₄	30.98	24.59	44.44
NE Challenge			
No Challenge	30.71	24.39	44.91
Challenge	30.62	24.60	44.79
P value			
Diet	0.250	0.806	0.601
NE Challenge	0.711	0.385	0.595
Interaction	0.149	0.733	0.341

Values are expressed as the mean and pooled SEM (n = 6). Means in a row with no common superscript are significantly different ($P < 0.05$). Lowercase letters indicate significantly different interactions and challenge effects, uppercase letters indicate significantly different dietary effects. SEM: standard error of the mean; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. The abbreviations of the treatment groups are the same as the previous ones.

eicosadienoic acid and dihomo- α -linolenic acid ratios, confirming that zinc plays an active role in the synthesis of PUFA, which has the same function as other antioxidant substances such as glycerol monolaurate (Valentini et al. 2020). The NE challenge down-regulated the proportion of dihomo- α -linolenic acid in chicken meat, suggesting that NE induced lipid peroxidation and affected the PUFA biosynthetic pathway, which is consistent with the untargeted metabolomics analysis. The NE challenge also reduced the arachidic acid level in chicken meat, suggesting an effect of immune challenge on fatty acid anabolism. Non-targeted metabolomic analyses were also used for the first time to study the effect of NE challenge on chicken meat, with quality control analyses showing the validity and stability of the assay results, while PCA and RSD analyses demonstrated the reproducibility and reliability of the

data. Notably, the results of the non-targeted metabolomics analysis made evident that fatty acid metabolic synthesis was affected by both, NE challenge and zinc, while a range of amino acid metabolic pathways were also shown to be affected, and these pathways also play important roles in meat quality regulation. We may refine these studies in the future.

An earlier study by Wu et al. (2018) has shown that NE leads to concurrent liver inflammation and affects liver function, thus NE might alter the fatty acid composition of chicken meat by affecting lipid metabolism (Wu et al. 2018). Blood biochemicals such as GLU, TG, TC, LDL and HDL levels reflect the metabolism and absorption of carbohydrates, proteins and fats in broilers (Hu et al., 2021). The amount of TCHO in the blood reflects the level of lipids, and abnormal increases in TCHO have been linked to the development of a large number of diseases, including inflammation of the liver and heart and blood vessel disease (Lauridsen et al. 2018). The levels of GLU and TG in the serum are regulated by diet and could indicate the fat deposition condition in poultry (Velasco et al. 2010). LDL contains a higher content of cholesterol and excessive LDL increases the risk of vascular and cardiac diseases, while HDL could prevent similar diseases by participating in reverse cholesterol transport (Hernández et al. 2019). In the present study, the NE challenge increased liver and serum TCHO levels, decreased TG levels, and increased serum LDL level, suggesting that NE induced abnormalities in liver lipid metabolism and increased the risk of lipid metabolism-related diseases. In contrast, zinc glycinate decreased serum GLU and increased TG level. These results might suggest that zinc supplementation enhances cholesterol catabolism and lipid metabolism of liver, which was also confirmed in data on fatty acid synthesis and oxidative gene expression in the liver. The ACC is a key enzyme in fatty acid synthesis, SREBP-1C is an important cholesterol synthesis regulatory protein, and increased PPAR- α and ACOX1 gene expression promotes fatty acid oxidation (Kim et al. 2017). The present study demonstrated that NE challenge resulted in downregulation of PPAR- α and ACOX1 gene expression, as well as upregulation of SREBP-1C, matching a previous result (Zhou et al. 2016). In addition, both sources of zinc could inhibit fatty acid synthesis by down-regulating gene expression in the liver ACC. Since the broiler diet is a typical high energy diet, the inhibition of the ACC pathway would prevent excessive fat deposition in the liver by regulating lipid metabolism disorders (Pang et al. 2021). These results suggest that NE leads to dysfunction of liver lipid metabolism and induces hyperlipidemia in broilers, and dietary

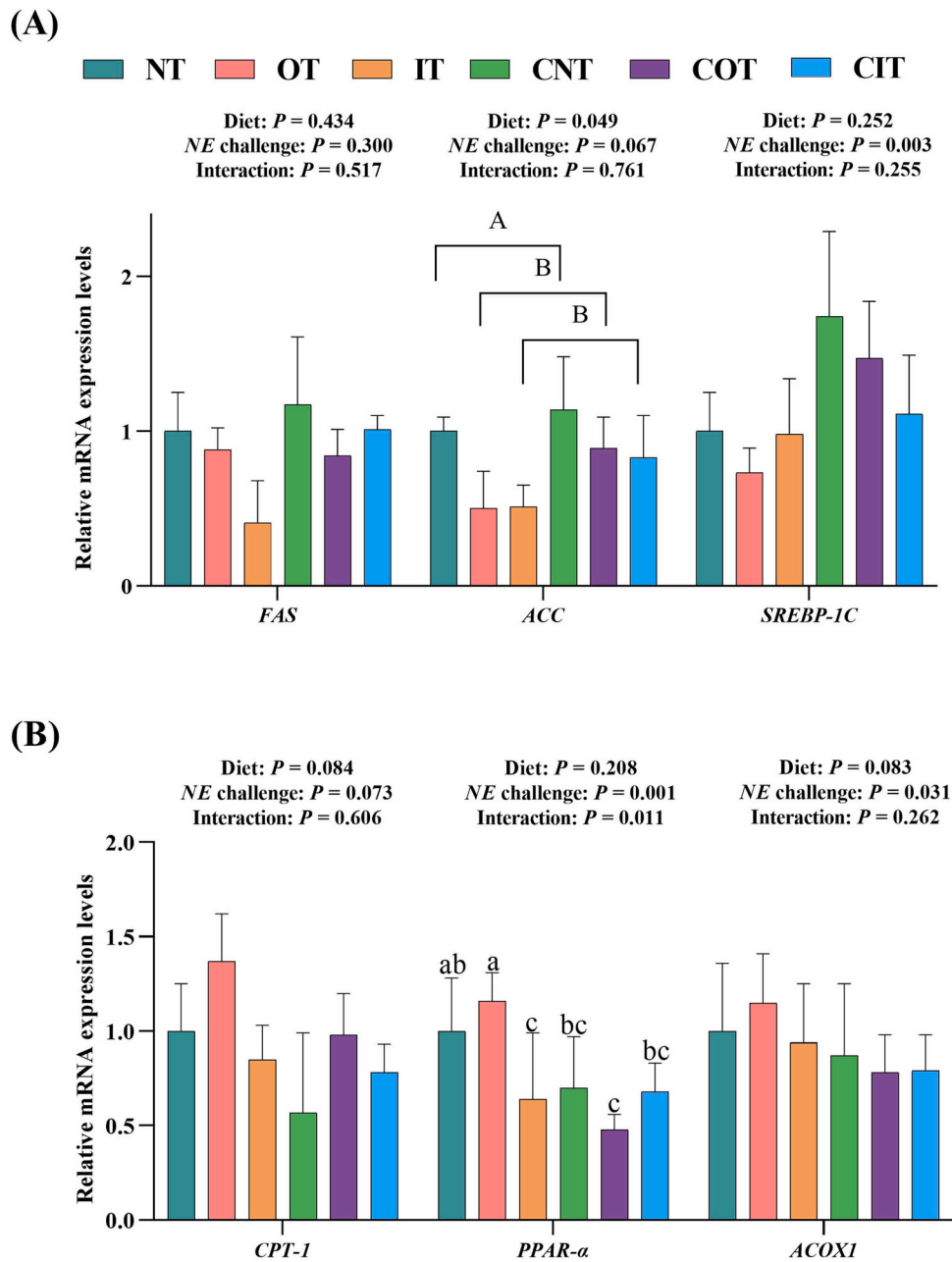


Fig. 4. Effect of dietary zinc on the gene expression levels of fatty acid synthesis (A) and oxidation (B) in the liver of necrotic enteritis challenged broilers. Means with no common superscripts differ significantly ($P < 0.05$), with lowercase letters indicating significantly different interactions and uppercase letters indicating significantly different dietary effects. FAS = fatty acid synthase; ACC = acetyl-CoA carboxylase; SREBP-1C = sterol regulatory element binding protein 1c; CPT-1 = carnitine palmitoyl transferase 1; PPAR- α = peroxisome proliferator activated receptor-alpha; ACOX1 = acyl CoA oxidase 1. The abbreviations of the treatment groups are the same as the previous ones.

zinc has a beneficial effect on lipid metabolism.

Conclusion

The current study demonstrated that the NE challenge affected carcass characteristics, exacerbated lipid peroxidation and altered the fatty acid composition of chicken meat, ultimately leading to a decrease in meat quality. Both zinc sources examined improved meat quality by modifying meat color and lipid peroxidation. The regulation of meat quality is related to the function of zinc and NE challenge through alteration of lipid metabolism.

CRediT authorship contribution statement

Chuanpi Xiao: Writing – original draft, Methodology, Investigation, Funding acquisition. **Xue Pan:** Methodology. **Peng Sun:** Methodology. **Martine Schroyen:** Writing – review & editing, Supervision. **Luke Comer:** Writing – review & editing. **Nadia Everaert:** Writing – review & editing, Supervision. **Zhigang Song:** Supervision.

Disclosures

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work presented in the manuscript entitled “Zinc improves meat quality

by modulating lipid metabolism in necrotic enteritis challenged broilers”.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.psj.2025.106086](https://doi.org/10.1016/j.psj.2025.106086).

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