

# Dietary Grape Seed Extract, Onion Peel Extract or Rosemary Extract supplementation alleviates Diquat-induced Restrained Growth and Oxidative Stress of Lohmann Chicks

**Man Wang**

Southwest University of Science and Technology

**Zongze He**

Southwest University of Science and Technology

**Zhaolong Xiong**

Southwest University of Science and Technology

**Hongwei Liu**

Southwest University of Science and Technology

**Xiang Zhou**

Southwest University of Science and Technology

**Jian He** (✉ [hejiantougao110@126.com](mailto:hejiantougao110@126.com))

Southwest University of Science and Technology

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## Research Article

**Keywords:** Antioxidant capacity, Grape seed extract, Onion peel extract, Rosemary extract, Lohmann chicks

**Posted Date:** November 11th, 2022

**DOI:** <https://doi.org/10.21203/rs.3.rs-2249118/v1>

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# Abstract

The present study was carried out to evaluate the *grape seed extract*, *onion peel extract* and *rosemary extract* on Diquat-induced restrained growth and oxidative stress in Lohmann chicks. A total of 200 Lohmann chicks, one day old, were assigned randomly into 5 diets: the positive control (PC) group, the negative control (NC) group, the grape seed extract (GSE) 100 mg/kg supplementing diet, the onion peel extract (OPE) 100 mg/kg supplementing diet and the rosemary extract (ROE) 100 mg/kg supplementing diet. During the first 7 days of trial, compared with NC and PC groups, the GSE group enhanced average daily feed intake ( $P < 0.05$ ). On day 8, all chicks were intraperitoneally injected with diquat except PC which was injected with normal saline. Diquat injection decreased the growth performance ( $P < 0.05$ ), increased platelet volume distribution width (PWD) concentration ( $P < 0.05$ ), increased malondialdehyde (MDA) concentration and activities of alanine aminotransferase (ALT) in the serum of chicks ( $P < 0.05$ ), decreased total protein (TP), albumin (ALB), globulin (GLB) concentration, activities of superoxide dismutase (SOD) and glutathione S-transferase (GST) in the serum ( $P < 0.05$ ), increased MDA concentration and decreased GST activities in the liver ( $P < 0.05$ ). From d 8 to 21, lower average daily gain (ADG) was observed in NC group than other groups ( $P < 0.05$ ). Compared with NC group, GSE decreased ALT activities, MDA, RDW and PDW concentration ( $P < 0.05$ ), increased SOD, GST activities ( $P < 0.05$ ). Compared with NC group, ROE decreased ALT activities and MDA concentration ( $P < 0.05$ ). Compared with NC group, the OPE group decreased ALT activities, MDA, RDW and PDW concentration ( $P < 0.05$ ), increased SOD activities ( $P < 0.05$ ) of chicks. These results suggest that supplementing antioxidants in diets alleviated oxidative stress in chicks challenged by improve antioxidant capacity and liver function.

## Introduction

Stress susceptibility of chicks is a major problem in the modern intensive poultry industry. The dynamic balance of the production and removal of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in chicks is broken, resulting in ROS and RNS not being cleared by the body in time, and excessive accumulation will damage the body, which will cause chicks reduced health and productivity (Santos-Sánchez et al. 2019). Diquat is currently the commonly used method for inducing oxidative stress in poultry. Diquat directly produces  $O_2$ ,  $-OH$ ,  $H_2O_2$  and other substances with strong oxidizing and cytotoxicity in the body (Xun et al. 2020). It is more direct and efficient to simulate the oxidative damage process of poultry in poultry production.

The body's antioxidant network system can be divided into two parts, antioxidant enzymes regulated by the antioxidant Keap1-Nrf2/ARE signaling pathway, and in vitro antioxidants with antioxidant function (Taleb et al. 2018). Plant extracts contain a higher content of plant polyphenols, the phenolic hydroxyl groups release highly active H ions to eliminate ROS in the body and protect the body from oxidative damage (Ognik et al. 2016). Rosemary extract (ROE) is mainly composed of aromatic essential oils, diterpenes, flavonoids, and fenacs. Some studies have found that the antioxidant function of ROE is carnosol, carnosic acid and rosmarinic acid in diterpenoids. Its antioxidant function is to eliminate ROS such as  $O_2$  and  $-OH$  through activated H ions in phenolic hydroxyl groups, reduce the accumulation of

ROS, block the process of lipid peroxidation, and prevent lipid peroxidation. Simultaneously combining metal ions reduces catalytic oxidation reactions during oxidative stress (Manafi et al. 2014; Radwan et al. 2008; Soltani et al. 2016). The main bioactive components in OPE are flavonoids and polyphenols, among which flavonoids include flavonols and derivatives up to 80%, and small amounts of flavonoids and flavonols, it has antioxidant, bactericidal, antibacterial, and immunity-boosting effects (Han et al. 2021). GSE has biologically active substances mainly grape polyphenols, also known as proanthocyanidins, which are present in grape seeds and grape skins, especially in grape seeds, which can be as high as 8% (Sogut and Seydim 2018). The main bioflavonoids in the grape polyphenols are 85%, which are composed of flavanol monomers and complexes. The biological activity of grape polyphenols comes from highly active phenolic hydroxy groups and conjugated double bonds, the reducing H provided by phenolic hydroxy groups, and the double bond structure has an efficient scavenging effect on ROS such as H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub>, -OH, etc (Guo et al. 2020). Our recent work has successfully induced oxidative stress in Lohmann chicks at 14 days of age by administrating diquat intraperitoneally, and has found that the diquat-induced oxidative stress resulted in serious liver damage, ultimately leading to the inferior growth performance of Lohmann chicks (Wang et al. 2022). This study was therefore conducted to explore the protective effects of dietary grape seed extract, onion peel extract or rosemary supplementation on the growth performance, antioxidant status, and liver function of Lohmann chicken in a model of diquat-induced oxidative stress, and the acquired results would provide valuable reference for alleviating oxidative stress occurred in Lohmann chick production.

## Materials And Methods

All animals were cared for as outlined in the Guide for the Care and Use of Animals in Research and Teaching Consortium. In this experiment, the grape seed extract, rosemary extract, and onion skin extract were provided by Nanjing Zhenweikang Biotechnology Co., Ltd., and extracted according to 10:1. Diquat was purchased from Sigma-Aldrich LLC., the 1-day-old Lohmann chicks was purchased from Sichuan Shengdile Village Ecological Food Co., Ltd.

## Animals And Housing

A total of two hundred one-day-old Lohmann chicks with a similar initial body weight (BW) of  $38.76 \pm 0.29$  g were used in a completely randomized design and were randomly allocated to one of the following five groups for a 21-day feeding experimental trial: 1) physiological saline-challenged chicks fed a basal diet (PC group); 2) diquat-challenged chicks fed a basal diet (NC group); 3) diquat-challenged chicks fed a basal diet supplemented with 100 mg/kg of grape seed extract (GSE group); 4) diquat-challenged chicks fed a basal diet supplemented with 100 mg/kg of onion peel extract (OPE group); 5) diquat-challenged chicks fed a basal diet supplemented with 100 mg/kg of rosemary extract (ROE group). Each experimental group consisted of five replicates (cages) of eight birds each. The diquat was dissolved in 0.86% physiological saline to prepare 10 mg/mL diquat solution prior to injection. At 8 days of age after

weighing, chicks were administered intraperitoneally with the diquat solution at a dosage of 1 mL/kg of BW or an equal amount of vehicle (0.86% physiological saline, control group).

## **Experimental Design And Diets**

The basal diets were formulated to meet or exceed the Layers Management guide (Lohmann brown-classic). The ingredients and nutrient composition of the basal diets were listed in Table 1. Chicks were reared in steel cages with plastic floors in a temperature-controlled room on a light schedule of 23 h of light and 1 h of dark. All birds were allowed free access to water and were fed ad libitum on mash diet except during the periods of fasting, when only water was available. The temperature of chick house was maintained between 32°C to 34°C during the first week after hatching, and it was then decreased by 2°C to 3°C at one week interval until a final temperature of 26°C was achieved. The relative humidity was set at approximate 55%-65% thereafter.

Table 1  
Ingredient and nutrient composition of the basal diets (air dry basis)

Ingredients, %	Content
Corn	56.68
Soybean meal	25.50
Extruded soybean	5.00
Rice bran meal	5.00
Corn gluten meal	3.00
Soybean oil	0.80
Limestone	1.15
Dicalcium phosphate	1.90
Salt	0.20
L-lysine HCl 78%	0.17
DL-methionine	0.15
Chloride choline	0.10
Mildew preventive	0.05
chicken premix <sup>1</sup>	0.30
Total	100.00
Analyzed composition <sup>2</sup> , %	
ME, MJ/kg	12.15
CP	19.97
Ca	0.96
Total P	0.74
<sup>1</sup> Provided the following per kilogram of diet: 10,000 IU of vitamin A, 3,920 IU of vitamin D <sub>3</sub> , 70 IU of vitamin E, 2.8 mg of vitamin B <sub>1</sub> , 8.4 mg of vitamin B <sub>2</sub> , 3.5 mg of vitamin B <sub>6</sub> , 42 mg of nicotinic acid, 23 mg of D-pantothenic acid, 3.5 mg of folic acid, and 800 mg of choline, 40 mg of Fe, 15 mg of Cu, 100 mg of Zn, 100 mg of Mn, and 0.35 mg of Se.	
<sup>2</sup> Metabolizable energy is calculated according to the Chinese Feed Composition and Nutritional Value Table (30th Edition in 2019), and the rest are measured values.	
Measured values are the average values of triplicate measurements.	

## Sample Collection

One bird from each cage (replicate) with a BW close to the replicate mean (five birds for each treatment in total) was selected for sampling. Blood sample was collected from the left side of the vein after 12 h of fasting on day 22, to examine routine blood indices. After centrifugation (3 000×g for 15 min at 4°C), serum samples were collected and stored at – 20°C for the determination of serum biochemical and antioxidant indices. Birds were euthanized by cervical dislocation method and necropsied after blood sampling. Liver was then dissected free from vessels and surrounding tissues, rinsed off with an ice-cold phosphate buffer solution, surface-dried with filter-paper, and weighed to calculate absolute liver weight and relative liver weight, using the formula: relative liver weight (g/kg) = absolute liver weight/terminal BW. Then, the tissues of liver were frozen in liquid nitrogen, and stored at – 80°C until analysis.

## Growth Performance Determination

After overnight fasting, all birds were weighted on the mornings of d 8 and 22, and feed intake of each cage was monitored daily throughout the trial. These data were used to calculate average daily feed intake (ADFI), average daily gain (ADG) and feed efficiency (F/G).

## Routine Blood And Biochemical Indices

Eleven routine blood indices, white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV) and platelet volume distribution width (PDW), were measured by blood cell analyzer (Sysmex XT-1800, Japan). Five blood biochemical indices, alanine transaminase (ALT), aspartate transaminase (AST), total protein (TP), albumin (ALB), and globulin (GLB), were determined using an automatic biochemistry analyzer (Hitachi 7020, Japan).

## Antioxidant Indices

Antioxidant indices in the serum and liver, including superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), glutathione S-transferase (GST), malondialdehyde (MDA), and total antioxidant capacity (T-AOC), were measured by specific commercial assay kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions.

## Statistical analysis

Each replicate served as an experimental unit. Data were analyzed by one-way analysis of variance using SPSS (2008) statistical software (Ver.16.0 for windows, SPSS Inc., Chicago, IL, USA). Differences among the three experimental groups were examined using Tukey's multiple range test. The differences were

considered as statistically significant when  $P < 0.05$ . Results were presented as means with their pooled standard errors.

## Results

### Growth Performance

During the entire trial period, there were not any unexpected deaths. as shown in Table 2. From d 1 to 7, compared with the PC and NC groups, the GSE group significantly increased ADFI of chicks ( $P < 0.05$ ). From d 8 to 21, lower ADG were observed in NC group than other groups ( $P < 0.05$ ).

Table 2  
Effect of feeding grape seed extract, onion peel extract or rosemary extract on growth performance in chicks<sup>1</sup>

Items <sup>2</sup>	PC	NC	ROE	OPE	GSE	SEM	P-value
Initial BW, g	38.53	38.59	39.08	38.91	38.7	0.68	0.98
1 ~ 7 d							
ADFI, g/d	12.08 <sup>b</sup>	11.93 <sup>b</sup>	12.25 <sup>ab</sup>	12.55 <sup>ab</sup>	13.02 <sup>a</sup>	0.36	0.04
ADG, g/d	4.74	4.67	4.82	4.99	5.13	0.12	0.45
F/G	2.55	2.55	2.54	2.52	2.54	0.03	0.82
8 ~ 21 d							
ADFI, g/d	20.19	17.96	18.95	18.38	20.49	0.44	0.17
ADG, g/d	7.86 <sup>a</sup>	6.64 <sup>b</sup>	7.76 <sup>a</sup>	7.88 <sup>a</sup>	8.28 <sup>a</sup>	0.25	0.03
F/G	2.57	2.7	2.44	2.33	2.48	0.05	0.14

<sup>1</sup>This study used 200 Lohman chicks with an average initial body weight of  $38.76 \pm 0.29$  g. Each mean represents five replicate pens with ten birds/pen. Means within a row with different letters differ significantly ( $P < 0.05$ ).

<sup>2</sup>Initial BW = initial body weight; ADFI = average daily feed intake; ADG = average daily gain; F/G = ADFI/ADG. PC = positive control group; NC = negative control group; GSE = grape seed extract group; OPE = onion peel extract group; ROE = rosemary extract group.

### Routine blood indices

The routine blood indices of chicks are presented in Table 3. On d 22, compared with PC group, Diquat challenge increased blood PWD of chicks ( $P < 0.05$ ). Compared with NC group, the GSE and OPE group significantly decreased RDW and PDW concentration ( $P < 0.05$ ).

Table 3

Effect of grape seed extract, onion peel extract or rosemary extract on blood routine of chicks under oxidative stress<sup>1</sup>

Items <sup>2</sup>	PC	NC	ROE	OPE	GSE	SEM	P-value
WBC, 10 <sup>9</sup> /L	177.22	190.98	205.74	213.44	217.54	5.70	0.13
RBC, 10 <sup>9</sup> /L	1.61	1.87	1.90	2.18	2.16	0.08	0.16
HGB, g/L	82.60 <sup>b</sup>	89.40 <sup>ab</sup>	97.20 <sup>ab</sup>	109.60 <sup>a</sup>	110.00 <sup>a</sup>	3.56	0.04
HCT, %	21.48	25.24	25.11	29.16	28.72	1.10	0.16
MCV, fL	134.36	135.18	132.12	134.38	133.50	0.57	0.54
MCH, pg	51.56	49.16	50.90	50.50	50.92	0.62	0.82
MCHC, g/L	385.00	364.20	386.20	376.40	382.80	4.51	0.55
RDW, fL	10.44 <sup>ab</sup>	11.54 <sup>a</sup>	10.78 <sup>ab</sup>	9.26 <sup>b</sup>	9.10 <sup>b</sup>	0.31	0.04
PLT, 10 <sup>9</sup> /L	9.60	14.60	14.20	15.00	15.00	1.13	0.53
MPV, fL	5.68	6.26	6.40	5.72	5.76	0.12	0.08
PDW, %	18.72 <sup>b</sup>	19.46 <sup>a</sup>	19.42 <sup>a</sup>	18.60 <sup>b</sup>	18.78 <sup>b</sup>	0.14	0.04

<sup>1</sup>Each mean represents five replicate pens. Means within a row with different letters differ significantly ( $P < 0.05$ ).

<sup>2</sup> WBC = white blood cell count; RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; PLT = platelet; MPV = mean platelet volume; PDW = platelet volume distribution width. PC = positive control group; NC = negative control group; GSE = grape seed extract group; OPE = onion peel extract group; ROE = rosemary extract group.

## Liver Weight and Serum biochemical indices

The absolute liver weight (Fig. 1,  $P > 0.05$ ) did not differ among treatments. As shown in Table 4, Compared with PC group, diquat challenge increased ALT and AST activities ( $P < 0.05$ ). Compared with NC group, the OPE, ROE and GSE groups decreased ALT activities ( $P < 0.05$ ) in plasma of chicks.



Table 4

Effect of grape seed extract, onion peel extract or rosemary extract on serum biochemical indexes of chicks under oxidative stress<sup>1</sup>

Items <sup>2</sup>	PC	NC	ROE	OPE	GSE	SEM	P-value
ALT, U/L	4.22 <sup>c</sup>	6.73 <sup>a</sup>	5.46 <sup>b</sup>	5.31 <sup>b</sup>	5.81 <sup>b</sup>	0.21	< 0.01
AST, U/L	231.82 <sup>b</sup>	279.19 <sup>a</sup>	288.59 <sup>a</sup>	272.26 <sup>a</sup>	276.24 <sup>a</sup>	6.19	0.02
TP, g/L	33.11	28.60	29.90	27.54	31.21	0.69	0.06
ALB, g/L	14.93	12.92	13.67	12.55	13.99	0.29	0.08
GLB, g/L	18.17	15.67	16.23	14.99	17.22	0.43	0.14
<sup>1</sup> Each mean represents five replicate pens. Means within a row with different letters differ significantly ( $P < 0.05$ ).							
<sup>2</sup> ALT = alanine aminotransferase; AST = aspartate aminotransferase; TP = total protein; ALB = albumin; GLB = globulin. PC = positive control group; NC = negative control group; GSE = grape seed extract group; OPE = onion peel extract group; ROE = rosemary extract group.							

## Serum and liver antioxidant indices

The serum antioxidant indices of chicks are represented in Table 5. Compared with PC group, diquat challenge increased MDA concentration and decreased GST and SOD activities ( $P < 0.05$ ). Compared with NC group, the OPE group increased SOD activities ( $P < 0.05$ ) and decreased MDA concentration, the ROE group decreased MDA concentration, the GSE group increased SOD, GST activities ( $P < 0.05$ ) and decreased MDA concentration in plasma of chicks ( $P < 0.05$ ). The liver antioxidant indices are shown in Table 5. Compared with PC group, diquat challenge increased MDA concentration and decreased GST activities ( $P < 0.05$ ) in the liver. Compared with NC group, the OPE group increased SOD, GST activities ( $P < 0.05$ ) and decreased MDA concentration ( $P < 0.05$ ), ROE group decreased MDA concentration ( $P < 0.05$ ), GSE group increased SOD, GST activities ( $P < 0.05$ ) and decreased MDA concentration in liver of chicks ( $P < 0.05$ ).

Table 5

Effect of grape seed extract, onion peel extract or rosemary extract on antioxidant index of chicks under oxidative stress<sup>1</sup>

Items <sup>2</sup>	PC	NC	ROE	OPE	GSE	SEM	P-value
Serum							
SOD, U/mg	494.34 <sup>b</sup>	441.57 <sup>c</sup>	442.18 <sup>c</sup>	474.93 <sup>ab</sup>	533.77 <sup>a</sup>	8.48	< 0.01
MDA, nmol/mg	4.13 <sup>b</sup>	7.75 <sup>a</sup>	4.69 <sup>b</sup>	4.81 <sup>b</sup>	3.43 <sup>b</sup>	0.37	< 0.01
GSH-PX, µg/mg	516.04	432.53	530.38	605.27	595.39	29.27	0.36
GST, U/mg	14.93 <sup>a</sup>	12.92 <sup>b</sup>	13.67 <sup>b</sup>	12.55 <sup>b</sup>	13.99 <sup>a</sup>	0.29	< 0.01
T-AOC, U/mg	18.17	15.67	16.23	14.99	17.22	0.43	0.55
Liver							
SOD, U/mg	805.77 <sup>ab</sup>	650.70 <sup>b</sup>	703.13 <sup>b</sup>	1013.51 <sup>a</sup>	1035.91 <sup>a</sup>	49.35	0.02
MDA, nmol/mg	0.30 <sup>b</sup>	1.02 <sup>a</sup>	0.29 <sup>b</sup>	0.21 <sup>b</sup>	0.24 <sup>b</sup>	0.07	< 0.01
GSH-PX, µg/mg	50.84	44.73	50.15	58.55	55.62	3.07	0.70
GST, U/mg	30.73 <sup>ab</sup>	21.87 <sup>c</sup>	26.63 <sup>bc</sup>	35.14 <sup>ab</sup>	36.00 <sup>a</sup>	1.57	< 0.01
T-AOC, U/mg	0.13	0.11	0.12	0.14	0.15	0.05	0.07
<sup>1</sup> Each mean represents five replicate pens. Means within a row with different letters differ significantly ( $P < 0.05$ ).							
<sup>2</sup> SOD = superoxide dismutase; MDA = malondialdehyde; GSH-PX = glutathione peroxidase; GST = glutathione S-transferase; T-AOC = total antioxidant capacity. PC = positive control group; NC = negative control group; GSE = grape seed extract group; OPE = grape seed extract group; ROE = grape seed extract group.							

## Discussion

When poultry is under oxidative stress, the complex regulatory mechanism of the body will be activated, and the energy required for growth will be used to resist the oxidative damage caused by the body, resulting in the decline of the growth performance of the poultry, and even death in severe cases (Taleb et al. 2018). In production, antioxidant substances are often added to poultry diet or drinking water to prevent oxidative stress (Han and Song 2021). In scientific research, in order to study oxidative stress related experiments, hormones, fatty acids, Diquat, bacterial lipopolysaccharides, etc. are usually used to build oxidative stress models (Reckelhoff et al. 2019). Diquat-induced oxidative stress disrupts the body's oxidative defenses, causes liver and small intestine damage, triggers acute inflammation, and inhibits nutrient absorption, resulting in decreased animal growth performance (Chen et al. 2020). Research on

the effects of diquat-induced oxidative stress on livestock growth performance has focused on piglets (Azad et al. 2021; Cao et al. 2018; Doan et al. 2020). In this research, the intraperitoneal administration with diquat reduced ADG change rate during 8 ~ 21d in Lohmann chicks. Chen et al (2020) have found that the intraperitoneal administration with diquat reduced ADG and BW change rate during the 24-h post-challenge in broiler chicks, this is consistent with the results of this study. The present study also showed that intraperitoneal injection of diquat decreased the SOD, GST and GSH-Px activities of the plasma and liver, and increased the MDA concentration of the plasma and liver in the chicks. These results indicated that the oxidative stress model induced by diquat injection was successful, which is consistent with our previous studies (Wang et al. 2022). Some studies have pointed out that the most likely reason for the decline in growth performance is that diquat-induced oxidative stress generates ROS, which leads to lipid peroxidation, increases the permeability of cell membranes, affects the function of cells, and leads to mucosal damage and infection in the digestive tract (Cao et al. 2019; Doan et al. 2020). When the digestion and absorption function of animals is reduced, the functional cells related to digestion and absorption will die rapidly, and the animals will experience symptoms such as vomiting, diarrhea, and anorexia, which will eventually lead to a decrease in feed intake and daily gain, and an increase in feed-to-weight ratio (Sun et al. 2021).

GSE contains proanthocyanidins, grape polyphenols, bioflavonoids and other biologically active substances, which have various effects such as scavenging free radicals, anti-oxidation, anti-aging, antibacterial and anti-inflammatory, and enhancing immunity (Parandoosh et al. 2020). OPE contains high levels of flavonols and its derivatives, which are a class of flavonoid bioactive substances that can scavenge free radicals, sterilize and reduce inflammation (Masood et al. 2021). The most important bioactive substances in ROE are terpenes, phenols and acids, which have strong anti-tumor, anti-viral, antibacterial, and antioxidant effects (Pourbabaki et al. 2020). During the period without Diquat injection, compared with the blank group, the addition of grape seed extract to the chick's diet significantly improved the growth performance of chicks, which may be the main reason why grape seed extract improves the growth performance of oxidatively stressed chicks. During the period with Diquat injection, compared with the NC group, the addition of onion peel extract and rosemary extract to the chick's diet significantly improved the growth performance of chicks. However, the growth performance of chicks did not differ among the NC, OPE and ROE groups prior to diquat challenge, and this may be owing to the normal physiological status of chicks and the healthy feeding environment in this study.

Blood is an important part of the circulatory system and participates in every metabolic activity in the body (Grüneboom et al. 2019). The normal metabolic activities of the body are closely related to the routine blood indicators, which are extremely important to maintain the normal internal and external environment of the body (Congleton et al. 2006). Therefore, the number and morphological distribution of blood cells in the blood index are important basis for judging the health of the body (Simide et al. 2016). In our previous study, intraperitoneal injection of diquat at a dose of 10 mg/kg body weight significantly increased the blood PDW concentration, the levels of red and white blood cells also increased, but not significantly. This suggests that injection of diquat causes oxidative stress in chickens, resulting in infection and inflammatory responses in chicks. The PDW in the blood of the GSE and OPE groups were

lower than that of the NC group, indicating that GSE and OPE can rapidly prevent the increase of PDW after Diquat caused infection and inflammation, and help the body fight infection and inflammation (Grüneboom et al. 2019). In this experiment, we found that the RBC, HGB, HCT index values in the blood of the PC group were lower than those of the other groups (injected with diquat). Studies have proven that under conditions of acute oxidative stress caused by diquat, animals had enhanced respiration, increased basal metabolic levels, and increased oxygen consumption, resulting in elevated RBC, HGB, and HCT (El-Deen et al. 1992). The HGB in the blood of the GSH group was significantly higher than that of the PC group. GSE, OPE and ROE could increase the oxygen-carrying capacity of the blood, provide more oxygen for respiration, relieve the hypoxia symptoms caused by Diquat, and help the chickens return to normal as soon as possible.

Diquat is widely considered as an effective chemical agent for inducing oxidative stress, which of the major target organ is the liver (Mao et al. 2014). In our previous study, intraperitoneal injection of diquat at a dose of 10 mg/kg body weight significantly increased the plasma ALT and AST activities. ALT and AST were mainly distributed in the liver cells. The concentrations of ALT and AST in serum reflect the degree of liver damage, and the higher the concentrations of ALT and AST, the more serious the liver damage (Qiao et al. 2020). When the phospholipid bilayer on the biomembrane in the liver cells is lipidated, the glycoprotein is damaged, the permeability of the biomembrane is enhanced, and the cells are apoptotic, causing the ALT and AST in the liver to enter the blood, and the ALT and AST in the blood increase (Jia et al. 2020). The present study showed that, compared with the PC group, the serum ALT and AST concentrations of the NC group were significantly increased. Under the condition of Diquat-induced oxidative stress, the NC group chickens developed oxidative stress symptoms of liver damage, which is consistent with the findings of Strange et al. The concentrations of TP, ALB and GLB in serum also reflect the protein synthesis function of the liver and the nutritional status of the body (Guo et al. 2022). This study showed that the concentrations of TP, ALB, and GLB in serum were lower in the NC group than in the PC group, and Diquat induced oxidative stress to damage liver function. Studies have shown that GSE, OPE and ROE have an improving effect on liver function (Çetin et al. 2008; Elhassaneen et al. 2014; Singletary et al. 1996), it is consistent with the results in this experiment.

When livestock and poultry undergo oxidative stress, the ROS produced in the animal exceeds the scavenging ability of the antioxidant enzyme system and non-enzymatic system in the body, and the antioxidant capacity of livestock and poultry will decrease (Taleb et al. 2018). The most direct reflection is that the concentration of antioxidant enzymes decreases, including SOD, GSH-Px, GST and other enzymes (Manafi et al. 2014). In this experimental study, antioxidant capacity of chicks decreased after Diquat injection. The injection of Diquat in chicks will induce a large amount of ROS produced by the body, breaking the balance of the normal antioxidant system (Taleb et al. 2018). Research showed that in the oxidative stress model of broiler chickens induced by cortisone, the content of MDA in serum, liver and muscle tissue was significantly increased, while SOD and GSH-Px were significantly reduced, which was similar to the results of this experiment (Chen et al. 2020). In animals under oxidative stress, unsaturated fatty acids are oxidized into lipid peroxides, which are finally decomposed to form MDA (Radwan et al. 2014). Therefore, the content of MDA in the body directly reflects the degree of lipid

peroxidation in the body, as well as the degree of oxidative damage (Han et al. 2021). In this experiment, the MDA content in serum and liver of NC group was significantly higher than that of PC group. Some scholars believe that the increase and decrease of antioxidant enzymes are the protective mechanism of the antioxidant enzyme system (Radwan et al. 2008). In a healthy state, antioxidant enzymes are maintained at a relatively constant, balanced, and low concentration level (Ognik et al. 2016). In the early stage of oxidative stress, the antioxidant enzyme system and non-enzymatic system in the body remove free radicals in the body and restore the body to normal (Doan et al. 2020). When the free radicals in the body exceed the scavenging ability of the antioxidant enzyme system and the non-enzyme system, the level of antioxidant enzymes in the body will be reduced, and the body will be in a state of oxidative stress (Reckelhoff et al. 2019). Many plant extracts and plant products have been shown to have significant antioxidant activity. It is well known the plant extract constituents could influence the differences in the antioxidant ability of plant extracts (Radwan et al. 2008). In this experiment, the antioxidant activity and free radical scavenging properties of each plant extract were different, among which GSE had the best antioxidant activity. Studies have shown that dried grape seeds (ORAC values 108130  $\mu\text{m TE}/100\text{ g}$ ) and rosemary (ORAC values 165280  $\mu\text{m TE}/100\text{ g}$ ) have higher antioxidant capacity in vitro, and dried onion (ORAC values 4289  $\mu\text{m TE}/100\text{ g}$ ) had lower antioxidant capacity (Brewer et al. 2011; Celano et al. 2021). The antioxidant capacity of these plant extracts in animals can also be found in the literature (Han and Song 2021; Manafi et al. 2014; Sogut and Seydim 2018), but none of the papers have compared the antioxidant capacity of these three in vivo simultaneously.

In conclusion, the acquired results in this study suggested that dietary supplementation with grape seed extract, onion peel extract or rosemary extract can improve growth performance, antioxidant status in plasma and liver, and liver function of Lohmann chicks subjected to diquat-induced oxidative stress. Additionally, based on the results of this study, under normal or oxidative stress conditions, adding grape seed extract to the diet can better improve the growth performance and antioxidant capacity of chicks compared with the OPE and ROE groups.

## Declarations

### Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

### Author ORCIDs

M. Wang: 0000-0002-1859-6581

Z.Z. He: 0000-0002-3660-1188

Z.L. Xiong: 0000-0002-9666-1166

H.W. Liu: 0000-0002-4000-2639

X. Zhou: 0000-0001-9781-3216

J. He: 0000-0002-7906-4132

### **Author contributions**

Man Wang, Jian He conceived and designed the research. Zhaolong Xiong, Hongwei Liu and Xiang Zhou acquisition of data. Man Wang, Zongze He and Zhaolong Xiong analyzed data, Man Wang interpreted the experimental results, prepared figures, and drafted, edited. Zongze He revised the manuscript. Man Wang and Jian HE approved the final version of the manuscript.

### **Acknowledgments**

We thank the staff of the Animal Health Breeding Team for their technical support in conducting the experiment and Xiaoling Yuan and Liyan Wang for their assistance during laboratory work.

### **Financial support statement**

This research received no specific grant from any funding agency, commercial or not-for-profit section.

### **Ethics approval and consent to participate**

This study was approved by the animal experiment was approved by the Ethics Committee on Animal Use of the Southwest University of Science and Technology.

### **Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### **Author details**

<sup>1</sup>School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang, Sichuan, 621010, P.R. China.

## **References**

1. Azad MAK, Wang H, Yang H, Bie T, Zhou SY, Guan GP (2021) Effects of dietary carboxymethyl pachyman on oxidative stress and inflammation in weaned piglets challenged with diquat. *Anim Feed Sci Tech* 276:114922.
2. Brewer MS (2011) Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Compr Rev Food Sci Food Saf* 10(4):221-247.
3. Cao ST, Shen ZJ, Wang CC, Zhang QH, Hong QH, He YH, Hu CH (2019) Resveratrol improves intestinal barrier function, alleviates mitochondrial dysfunction and induces mitophagy in diquat

- challenged piglets. *Food Funct* 10(1):344-354.
4. Cao ST, Wu H, Wang CC, Zhang QH, Jiao LF, Lin FH, Hu CH (2018) Diquat-induced oxidative stress increases intestinal permeability, impairs mitochondrial function, and triggers mitophagy in piglets. *J Anim Sci* 96(5):1795-1805.
  5. Celano R, Docimo T, Piccinelli AL, Gazzero P, Tucci M, Sanzo RD, Carabetta S, Campone L, Russo M, Rastrelli L (2021) Onion peel: Turning a food waste into a resource. *Antioxidants Basel* 10(304):1-18.
  6. Çetin A, Kaynar L, Kocyigit I, Hacıoglu SK, Saraymen R, Ozturk A, Sari I, Sagdic O (2008) Role of grape seed extract on methotrexate induced oxidative stress in rat liver. *Am J Chinese Medicine* 36(05):861-872.
  7. Chen YN, Chen YP, Zhang H, Wang T (2020) Pterostilbene as a protective antioxidant attenuates diquat-induced liver injury and oxidative stress in 21-day-old broiler chickens. *Poultry Sci* 99(6):3158-3167.
  8. Congleton JL, Wagner T (2006) Blood-chemistry indicators of nutritional status in juvenile salmonids. *J Fish Biology* 69(2):473-490.
  9. Doan N, Liu Y, Xiong X, Kim K, Ji P (2020) Organic selenium supplement partially alleviated diquat-induced oxidative insults and hepatic metabolic stress in nursery pigs. *Brit J Nutr* 124(1):23-33.
  10. El-Deen MAS, Rogers WE (1992) Acute toxicity and some hematological changes in grass carp exposed to diquat. *J Aquat Anim Health* 4(4):277-280.
  11. Elhassaneen YA, Elhady YAA (2014) Onion peel powder alleviate acrylamide-induced cytotoxicity and immunotoxicity in liver cell culture. *Life Sci J* 11(7):381-388.
  12. Grüneboom A, Hawwari I, Weidner D, Culemann S, Culemann S, Muller S, Henneberg S, Brenzel A, Merz S, Bornemann L, Zec K, Wuelling M, Kling L, Hasenberg M, Voortmann S, Lang S, Baum W, Ohs A, Kraff O, Quick HH, Jäger M, Landgraeber S, Dudda M, Danuser R, Stein JV, Rohde M, Gelse K, Garbe AI, Adamczyk A, Westendorf AM, Hoffmann D, Christiansen S, Engel DR, Vortkamp A, Krönke G, Herrmann M, Kamradt T, Schett G, Hasenberg A, Gunzer M (2019) A network of trans-cortical capillaries as mainstay for blood circulation in long bone. *Nat Metab* 1(2):236-250.
  13. Guo J, Yan WR, Tang JK, Jin X, Xue HH, Wang T, Sun QY, Liang ZX, Zhang LW (2022) Dietary phillygenin supplementation ameliorates aflatoxin B1-induced oxidative stress, inflammation, and apoptosis in chicken liver. *Ecotox Environ Safe* 236:113481.
  14. Guo Y, Huang J, Chen Y, Hou Q, Huang M (2020) Effect of grape seed extract combined with modified atmosphere packaging on the quality of roast chicken. *Poultry Sci* 99(3):1598-1605.
  15. Han HS, Song KB (2021) Antioxidant properties of watermelon (*Citrullus lanatus*) rind pectin films containing kiwifruit (*Actinidia chinensis*) peel extract and their application as chicken thigh packaging. *Food Packaging Shelf* 28:100636.
  16. Jia P, Ji S, Zhang H, Chen Y, Wang T (2020) Piceatannol ameliorates hepatic oxidative damage and mitochondrial dysfunction of weaned piglets challenged with diquat. *Animals* 10(7): 1239.
  17. Manafi M, Hedayati M, Yari M (2014) Application of rosemary (*Rosmarinus officinalis* L.) essence on chicks fed aflatoxin B1: Impacts on internal organ weights, biochemical traits and mortality. *Res*

Zoology 4(1):13-19.

18. Mao XB, Lv M, Yu B, He J, Zheng P, Yu J, Wang QY, Chen DW (2014) The effect of dietary tryptophan levels on oxidative stress of liver induced by diquat in weaned piglets. *J Anim Sci Biotechnol* 5(1):1-7.
19. Masood S, Rehman AU, Bashir S, Shazly ME, Imran M, Khalil P, Ifthikar F, Jaffar HM, Khursheed T (2021) Investigation of the anti-hyperglycemic and antioxidant effects of wheat bread supplemented with onion peel extract and onion powder in diabetic rats. *J Diabetes Metab Dis* 20(1):485-495.
20. Nadia R, Hassan RA, Qota EM, Fayek HM (2008) Effect of natural antioxidant on oxidative stability of eggs and productive and reproductive performance of laying hens. *Int J Poultry Sci* 7(2):134-150.
21. Ognik K, Cholewińska E, Sembratowicz I, Eugeniusz RG, Czech A (2016) The potential of using plant antioxidants to stimulate antioxidant mechanisms in poultry. *World Poultry Sci J* 72(2):291-298.
22. Parandoosh M, Yousefi R, Khorsandi H, Nikpayam O, Saidpour A, Babaei H (2020) The effects of grape seed extract (*Vitis vinifera*) supplement on inflammatory markers, neuropeptide Y, anthropometric measures, and appetite in obese or overweight individuals: A randomized clinical trial. *Phytother Res* 34(2):379-387.
23. Pourbabaki R, Khadem M, Samiei S, Hasanpour H, Shahtaheri SJ (2020) The protective effect of rosemary in mitigating oxidative stress induced by Chlorpyrifos in rat kidney. *J Health Saf Work* 10(2):24-29.
24. Qiao L, Dou XN, Yan SQ, Zhang BH, Xu CL (2020) Biogenic selenium nanoparticles synthesized by *Lactobacillus casei* ATCC 393 alleviate diquat-induced intestinal barrier dysfunction in C57BL/6 mice through their antioxidant activity. *Food Funct* 11(4):3020-3031.
25. Reckelhoff JF, Romero DG, Cardozo LLY (2019) Sex, oxidative stress, and hypertension: insights from animal models. *Physiol* 34(3):178-188.
26. Sánchez NFS, Salas-Coronado R, Villanueva C, Hernández-Carlos B (2019) Antioxidant compounds and their antioxidant mechanism. *Antioxid* 10:1-29.
27. Simide R, Richard S, Prévot-D'Alvise N, Miard T, Gaillard S (2016) Assessment of the accuracy of physiological blood indicators for the evaluation of stress, health status and welfare in Siberian sturgeon (*Acipenser baerii*) subject to chronic heat stress and dietary supplementation. *Int Aquat Res* 8(2):121-135.
28. Singletary KW (1996) Rosemary extract and carnosol stimulate rat liver glutathione-S-transferase and quinone reductase activities. *Cancer Lett* 100(1-2):139-144.
29. Sogut E, Seydim AC (2018) The effects of Chitosan and grape seed extract-based edible films on the quality of vacuum packaged chicken breast fillets. *Food Packaging Shelf* 18:13-20.
30. Soltani M, Tabeidian SA, Ghalamkari G, Adelijoo AH, Mohammadrezaei M, Fosoul SSAS (2016) Effect of dietary extract and dried areal parts of *Rosmarinus officinalis* on performance, immune responses and total serum antioxidant activity in broiler chicks. *Asian Pac J Trop Disease* 6(3):218-222.
31. Sun XJ, Piao LG, Jin HF, Nogoy KMC, Zhang JF, Sun B, Jin Y, Lee DH, Choi S, Li XZ (2021) Dietary glucose oxidase and/or catalase supplementation alleviates intestinal oxidative stress induced by



- diquat in weaned piglets. *Anim Sci J* 92(1):e13634.
32. Taleb A, Ahmad KA, Ihsan AH, Qu J, Lin N, Hezam K, Koju N, Hui L, Ding QL (2018) Antioxidant effects and mechanism of silymarin in oxidative stress induced cardiovascular diseases. *Biomed Pharmacother* 102:689-698.
  33. Wang M, Li M, Liu HW, He YQ, Xiong ZL, He J (2022) Effects of *Macleaya cordata* Extracts on Growth Performance and Immune Function of Immunosuppressed Laying Chicks. *Anim Husbandry Feed Sci* 1:16-21.
  34. Xun WJ, Fu QY, Hou GY, Shi LG, Gao T (2020) Protective effects of dietary resveratrol supplementation against oxidative stress in diquat-challenged piglets. *Ital J Anim Sci* 19(1):1523-1532.