Serum metabolomics reveal pathways associated with protective effect of ginsenoside Rg3 on immune stress

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ABSTRACT Our previous study has demonstrated that administration of ginsenoside Rg3 ameliorates immune stress by inhibiting inflammatory responses, reducing oxidative damage and upregulating mRNA expression of mTOR, SOD-1, and HO-1. However, the specific mechanism in relation to the protective effect of ginsenoside Rg3 on stressed broilers especially the metabolites alteration remains obscure. The present study aimed to investigate the underlined mechanism in relation to the pathogenesis and protective effect of ginsenoside Rg3 on stressed broilers using liquid chromatograph-mass spectrometry profiling. Eighteen broiler chicks were randomly allocated to 3 treatments: Control, Model and Rg3. Chickens in Rg3 group received intraperitoneally administered 1 mg/kg Rg3 2 h before LPS challenge. Then the broilers were intraperitoneally injection of 250 μg/kg LPS at the age of 12, 14, 33, and 35 d to induce immune stress. Control group was injected with an equivalent amount of sterile saline. At the end of the experiment, the serum was obtained for metabolomics analysis. The changes in serum metabolic profiles were investigated with the application of metabolomics approach. Distinct changes in metabolite patterns in serum were observed by orthogonal partial least square-discriminate analysis. In total, 35 metabolites were identified, among which 17 differential metabolites were found between Control and Model group, and 18 differential metabolites were identified between Model and Rg3 group. Metabolic pathway analysis revealed potential serum metabolites involved in oxidative stress and inflammation, degradation of lipid and protein in broiler chicks with immune stress. In addition, the protective effect of Rg3 on the stressed chicks may be largely mediated by BCAA metabolism, apoptosis and mTOR signaling pathway. These results suggested the potential biomarkers involved in pathogenesis and prevention of stress induced by *Escherichia coli* lipopolysaccharide.

Key words: ginsenoside, lipopolysaccharide, stress, metabolomics

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INTRODUCTION

Broilers usually suffer from pathogens including *Escherichia coli* (***E. coli***) and *Salmonella enterica*, which may release lipopolysaccharide (**LPS**) and induce inflammatory responses (Csernus et al., 2020; Geng et al., 2018). Excessive inflammation will lead to tissue damage and metabolic disorder of the neuro-endocrine system, decreasing growth performance, and causing large economic loss (Liu et al., 2015; Zheng et al., 2021). Strategies including management, nutritional manipulation, and herbs have been shown to be effective to alleviate immune stress and enhance growth performance (Liu et al., 2015; Han et al., 2020). However, the specific mechanism in relation to the effect of herbs on immune stress of broilers, especially the metabolites alteration remains obscure.

LPS is traditionally used to induce immune stress in broilers (Li et al., 2015; Liu et al., 2015). LPS promotes production of several proinflammatory cytokines and inflammatory mediators by activating TLR4 and downstream signaling pathway such as NF-κB (Xin et al., 2021). Ginsenoside Rg3 is one of the main constituents extracted from *Panax ginseng* C. A. Meyer and has been demonstrated to effectively relieve inflammation and

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oxidative damage (Shi et al., 2020; Gao et al., 2021). Our previous study has demonstrated that Rg3 can ameliorate immune stress by inhibiting inflammatory responses, reducing oxidative stress and upregulating mRNA expression of *mTOR, SOD-1*, and *HO-1* (Bi et al., 2022). As metabolomics has served a dominant role in investigating pathogenesis and identifying potential drug targets, it is increasingly used to explore changes of small-molecule metabolites in biological systems in mammals and birds (Shao et al., 2018; Li et al., 2019). In the present study, we investigated the underlined mechanism in relation to the pathogenesis and protective effect of ginsenoside Rg3 on stressed broilers using liquid chromatograph-mass spectrometry (**LC-MS**) profiling. Our results may reveal the potential biomarkers in the pathogenesis and the prevention of immune stress in broiler chicks.

MATERIALS AND METHODS

Birds

A total of 18 one-day-old Hongyu commercial broiler chicks (male) were purchased from Sichuan Lihua Poultry Co., Ltd. and separately housed into 3-layer cages. The room temperature was set at 32°C to 34°C for the first 7 d, then gradually decreased by 1°C every 2 d until, finally maintained at 26°C. All broilers had free access to feed and water. The Animal Care and Use Committee of Southwest University approved all animal experiments (permit number IACUC-20200701-01).

Reagents

Ginsenoside Rg3 (98.18%; $C_{42}H_{72}O_{13}$; molecular weight, 785.025) was purchased from Chengdu Purify Biotechnology Co. Ltd. (Chengdu, China). The majority of ginsenosides are dammarane-type saponins. According to the saponins, dammarane-type ginsenosides can be divided into 2 types, protopanaxadiol (**PPD**) and protopanaxatriol (**PPT**). Rg3 extracted from the root of *Panax ginseng* C.A Meyer. is PPD-type ginsenosides with sugar moieties attached to the β-OH at C-3 and/or C-20 in the aglycon PPD. LPS (L2880) from *E. coli* serotype O55:B5 was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

Experimental Design

Eighteen broiler chicks were randomly allocated to 3 treatments (Table 1). Chickens in Rg3 group received an intraperitoneally administered dose of 1 mg/kg body weight of Rg3 2 h before LPS challenge. Then the broilers were injected intraperitoneally with 250 μg/kg LPS at the age of 12, 14, 33, and 35 d to induce immune stress. Control group was injected with an equivalent amount of sterile saline. At the end of the experiment, the serum was obtained from blood samples after centrifugation and then stored at −80°C for metabolomics analysis.

Table 1. Experimental design.

Group	n	LPS	Drug
Control	6	0	0
Model	6	250 μg/kg	0
Rg3	6	250 μg/kg	1 mg/kg Rg3

Blood Serum Metabolites Collection

Serum samples were taken out from the −80°C refrigerator and thawed on ice. A total of 50 μL serum was taken and 200 μL of methanol and acetonitrile (1:1) were added. The mixture was ultrasonic oscillated for 10 min and placed in −20°C refrigerator for 1 h. After centrifugation at 14,000 *g* for 15 min at 4°C, 180 μL of the supernatant was taken into another tube and dried in a vacuum freeze dryer. The dried extract was re-dissolved with 60 μL acetonitrile and water (1:1) and ultrasonic oscillated for 10 min. After centrifugation at 14,000 *g* for 15 min at 4°C, 30 μL of the supernatant was taken for analysis by UPLC-Q-TOF/MS.

UPLC-Q-TOF/MS Analysis

The method was performed as previously reported with minor modifications (Cai et al., 2015). Chromatographic separation of the serum extract was performed on an UPLC-QTOF-MS system (1290, Agilent Technologies Inc., Los Angeles, CA). The UPLC system was equipped with an Weaters Acquity UPLC BEH Amide column (2.1 mm × 100 mm, 1.7 μm; Waters Corp., MA). Mobile phases A was ultrapure water (contains 25 mM ammonium acetate and 25 mM ammonia) and mobile phases B was acetonitrile. The solvent gradient elution was set as follows: 0 to 0.5 min, 5% A, 95% B; 0.5 to 7 min, 35% A, 65% B; 7 to 9 min, 60% A, 40% B; 9 to 12 min, 5% A, 95% B. The injection volume was 2 μL, and the flow rate was set to 500 μL/min. The UPLC system was connected to a high-resolution accurate-mass spectrometer (Triple TOF 5600+, Sciex). The MS conditions were set as follows: 5.5 kV for positive and −4.5 kV for negative, mass spectrometer voltage; 60 V, decluttering voltage; 650°C, EFI ion source temperature; 9 L/min, drying gas flow rate; 60 psi, ion source gas1 (Gas1); 60 psi, ion source gas2 (Gas2); 30 psi, curtain gas (CUR). The extraction and analysis of metabolites were assisted by the Allwegene Technology Co., Ltd. (Beijing, China).

Statistics Analysis

The basic data analysis of metabolome analysis involving the peak number, sample name, and normalized peak area were fed to R package metaX for orthogonal partial least square-discriminate analysis (**OPLS-DA**), and to screen out metabolites with significant differences. The variable importance in the projection (**VIP**) values was calculated in the OPLS-DA model.

The R package MetaboAnalystR was used to generate receiver operating characteristic (**ROC**) curves. In addition, commercial databases including Kyoto Encyclopedia of Genes and Genomes (**KEGG**, http://www.kegg.jp) and MetaboAnalyst (http://www.metaboanalyst.ca/) were utilized to search for metabolic pathways. The P value and the pathway impact value are calculated using enrichment and pathway topology analysis, respectively. The influenced metabolic pathway was set as a pathway impact value ≥ 0.25 or $P < 0.05$. Correlation analysis between serum metabolites and clinical parameters (Bi et al., 2022) was performed using Spearman's rank correlation, and P value < 0.05 was considered as significantly associated metabolites. The correlation heatmap was generated using R program "heatmap" package and then was visualized by Cytoscape (Version 3.2.1) (Mateo-Otero et al., 2021).

RESULTS

Changes of Metabolite Levels

After data normalization, the score plots of OPLS-DA (Figure 1A) showed a clear discrimination between Model and Control without any overlap, demonstrating a robust metabolic difference between the 2 groups. Permutation test on the OPLS-DA model ($R^2Y = 0.996$, $Q^2 = 0.219$) confirmed that the model did not over-fit (Figure 1B). On the basis of VIP > 1 values derived from the OPLS-DA model and the P-value < 0.05, seventeen differential metabolites were obtained from the comparison Model vs. Control. Seven metabolites were upregulated such as Phenyl lactic acid, while ten metabolites were downregulated including Biliverdin and alpha-ketoglutarate. The significantly altered metabolites were shown in Table 2. Heatmap visualization (Figure 1C) of the potential candidate of importance showed distinct segregation between the Model and Control groups. As shown in Figure 1D, there was a clear discrimination between Rg3 and Model in the score plots of OPLS-DA, demonstrating a robust metabolic difference between the 2 groups. Permutation test on the OPLS-DA model ($R^2Y = 0.993$, $Q^2 = 0.327$) confirmed that the model did not over-fit (Figure 1E). A total of 18 differential metabolites were obtained from the comparison Rg3 vs. Model on the basis of VIP > 1 and the P-value < 0.05. Fifteen metabolites were upregulated, such as guanosine, γ-Glutamyl cysteine, L-valine and L-leucine, while 3 metabolites were downregulated, such as 1-

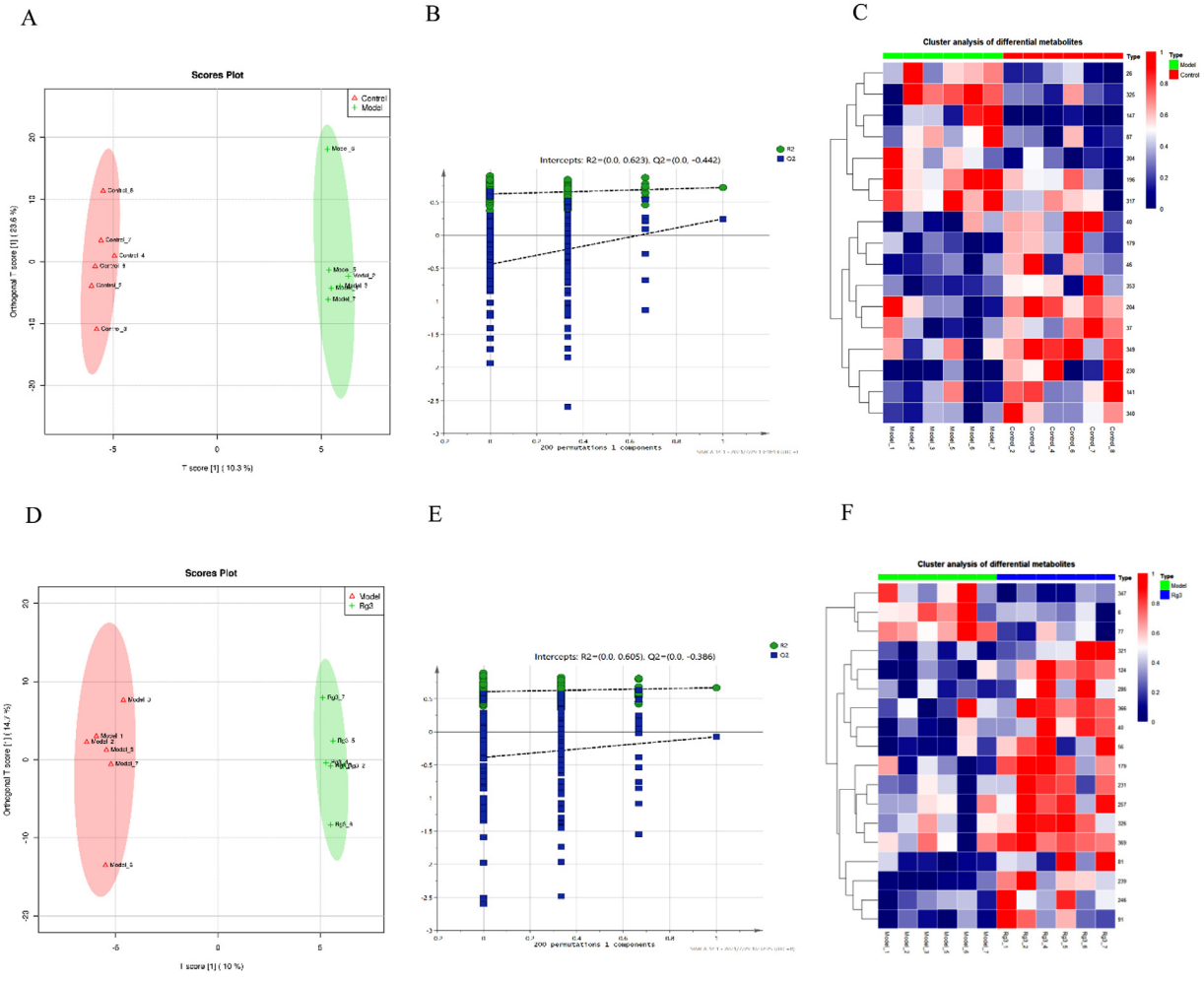


Figure 1. Analysis of metabolic alterations of the Control, Model, and Rg3 samples. (A) Orthogonal partial least squares-discriminate analysis (OPLS-DA) score plots of metabolite profile; (B) Permutation test on the OPLS-DA model and (C) Hierarchical clustering of differential metabolites in the comparison Model vs. Control. (D) OPLS-DA score plots of metabolite profile; (E) Permutation test on the OPLS-DA model and (F) Hierarchical clustering of differential metabolites in the comparison Rg3 vs. Model.

Table 2. Differential metabolites in Model birds compared to Controls.

Metabolite	VIP	FC (Model vs. Control)	*P* value
Biotin-XX hydrazide	1.57	0.13	0.022
N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid	1.80	0.56	0.048
Biliverdin	1.78	0.61	0.048
Ser-Arg	1.77	0.68	0.022
Stearoyl carnitine	1.78	0.69	0.030
1-Hexadecyl-sn-glycero-3-phosphocholine	1.97	0.71	0.032
4-Hydroxycinnamic acid (L-phenylalanine methyl ester) amide	2.01	0.73	0.035
alpha-ketoglutarate	1.94	0.78	0.038
Taurolithocholic acid	1.82	0.81	0.028
Apigenin 7-glucoside	1.73	0.86	0.040
Glutaric acid	1.79	1.17	0.049
N-Acetylmannosamine	1.92	1.20	0.039
3-Acetoxypyridine	1.75	1.21	0.043
3-Phenylpropanoic acid	2.31	1.21	0.009
PC(16:0/16:0)	1.89	1.32	0.035
Myristoleic acid	1.54	1.38	0.021
Phenyllactic acid	2.16	2.62	0.019

Abbreviations: FC, fold change; VIP, variable importance in the projection.

The fold change was calculated by comparing the values to those obtained on control.

The VIP values were obtained by OPLS-DA analysis.

Hexadecanoyl-sn-glycero-3-phosphoethanolamine and sphingosine. As shown in Figure 1F, red dots in the heatmap indicate the upregulated metabolites while blue dots indicate the downregulated metabolites in the comparison Rg3 vs. Model. The detailed information of significantly altered metabolites was listed in Table 3. The ROC curve of metabolic abnormalities identified between Control and Model are shown in Figure 2. All of the metabolites showed good discriminant value (ROC > 0.8). The ROC curve of metabolic abnormalities identified between Rg3 and Model are shown in Figure 3. Almost all of the metabolites (except for 231: ROC = 0.778) showed good discriminant value (ROC > 0.8).

Table 3. Differential metabolites in Rg3 compared to Model.

Metabolite	VIP	FC (Rg3 vs. Model)	*P* value
Guanosine	2.13	1.88	0.018
Cytosine	2.74	1.65	0.000
Bisindolylmaleimide IV	2.00	1.57	0.035
Diethanolamine	2.02	1.39	0.028
Cytidine	2.17	1.39	0.012
Gamma-Glutamylcysteine	1.93	1.37	0.030
1-Hexadecyl-sn-glycero-3-phosphocholine	2.37	1.37	0.006
Edaravone	1.93	1.33	0.043
Nicotinamide	1.84	1.27	0.046
L-Valine	2.51	1.26	0.003
"Pentanol, 5-amino-"	2.21	1.25	0.016
Val-Tyr	2.16	1.25	0.021
Uracil	1.82	1.23	0.044
Apigenin 7-glucoside	2.14	1.20	0.012
L-Leucine	1.93	1.18	0.039
1-Hexadecanoyl-sn-glycero-3-phosphoethanolamine	1.89	0.82	0.031
Enterostatin human	2.40	0.72	0.005
Sphingosine	2.18	0.64	0.015

The fold change was calculated by comparing the values to those obtained on model.

KEGG Analysis on Potential Metabolic Pathways

As shown in Figure 4, seven metabolic pathways were detected as potential metabolic pathways in relation to the protective effect of Rg3 on stressed broiler chicks. Among these pathways, mTOR signaling pathway and apoptosis were remarkedly altered ($P < 0.05$) with high impact values (Impact = 0.25). Valine, leucine, and isoleucine biosynthesis as well as valine, leucine, and isoleucine degradation were also significantly changed ($P < 0.05$) but with a low impact value (Impact < 0.1). Other obviously altered pathways include Pantothenate and CoA biosynthesis and Aminoacyl-tRNA biosynthesis ($P < 0.05$).

Correlations Between Biochemical Indicators and Potential Biomarkers

Seventeen potential biomarkers in the comparison Model vs. Control and biochemical indicators were used for the Pearson correlation analysis. As depicted in Figure 5A, the body weight was positively correlated 59% of the potential biomarkers and negatively correlated with 41% of the potential biomarkers. The body temperature and stress-related hormone (CORT and ACTH) were positively correlated with 41% of the potential biomarkers and negatively correlated with 59% of the potential biomarkers. In addition, the indicators of inflammatory response (IL-1β, IL-6, TNF-α, iNOS, and NO) and oxidative stress (MDA and carbonyl) were positively correlated with 41% of the potential biomarkers, while most antioxidant factors (T-AOC, T-SOD, GSH-PX, and GSH) were found to be negatively correlated with 41% of the potential biomarkers.

Eighteen potential biomarkers of Rg3 against Model and biochemical indicators were used for the Pearson correlation analysis. As shown in Figure 5B, the body weight was positively correlated with 83% of the potential biomarkers while the body temperature and stress-related hormone (CORT and ACTH) were negatively correlate with 83% of the potential biomarkers. In addition, the indicators of most inflammatory response (IL-6, TNF-α, iNOS) and oxidative stress (MDA and carbonyl) were negatively correlated with 83% of the potential biomarkers, while most antioxidant factors (T-AOC, T-SOD, GSH-PX, and GSH) were found to be positively correlated with 83% of the potential biomarkers.

DISCUSSION

Our previous study demonstrated that ginsenoside Rg3 improved the growth performance, shortened the duration of fever and the maximum body temperature, and decreased the serum CORT and ACTH, as well as inhibited the production of pro-inflammatory cytokines and inflammatory mediators and reduced the oxidative damage in broilers with stress challenged by LPS

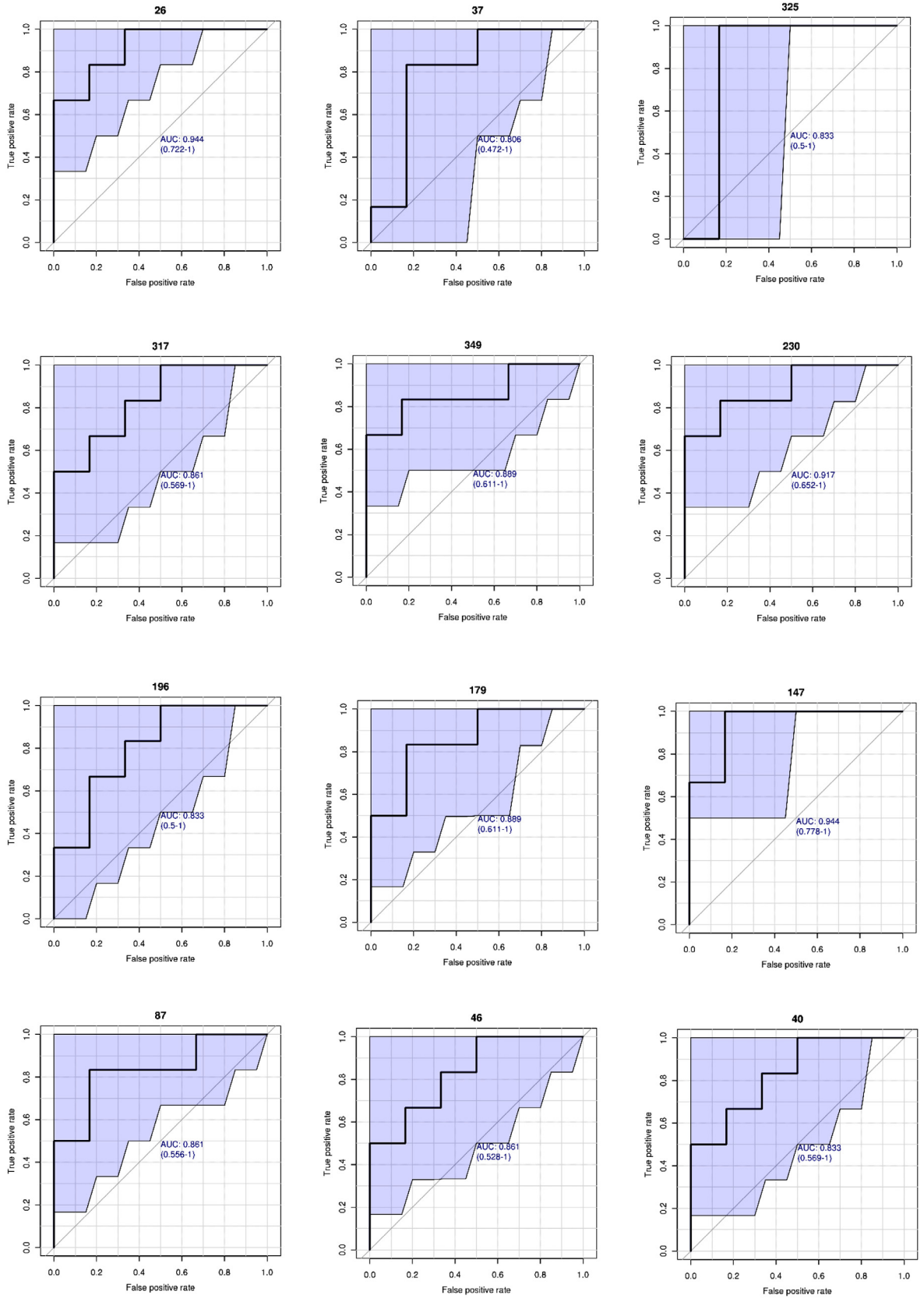
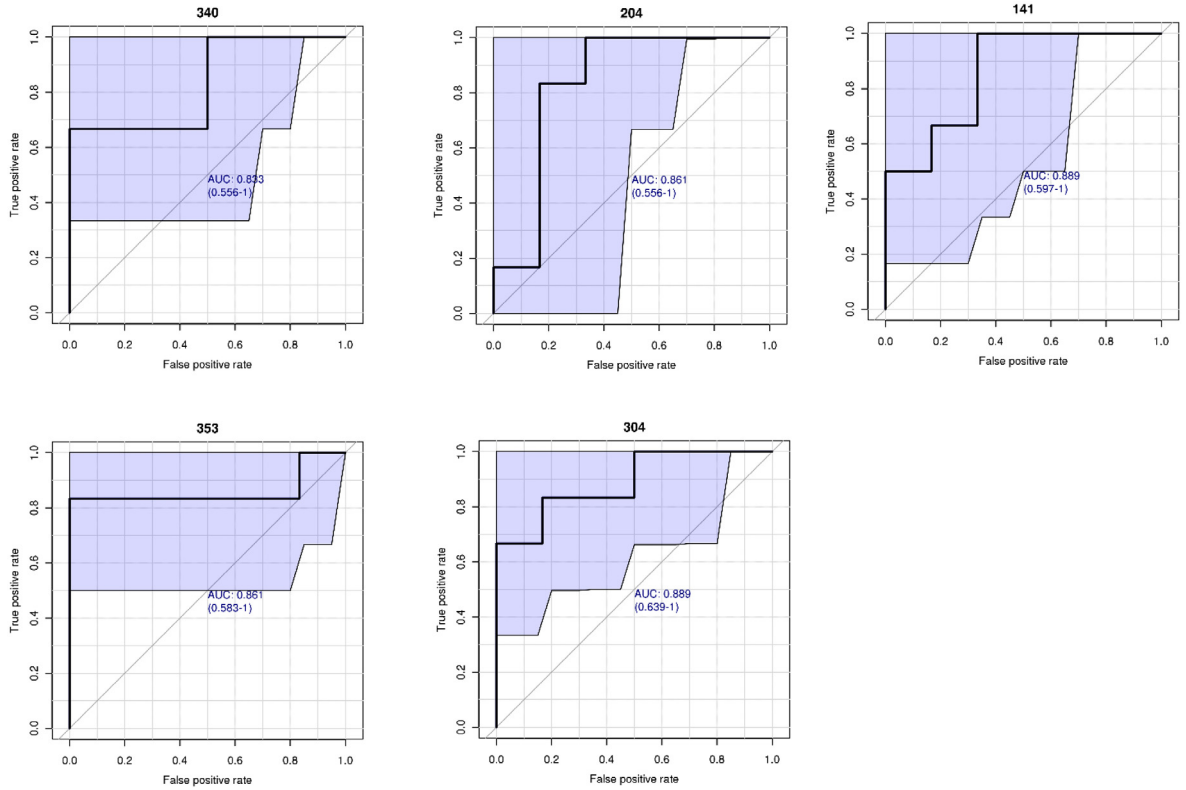


Figure 2. ROC curve analysis for metabolites in the comparison Model vs. Control. 230: Biotin-XX hydrazide; 141: N-Tris(hydroxymethyl) methyl-2-aminoethanesulfonic acid; 46: Biliverdin; 340: Ser-Arg; 349: Stearoyl carnitine; 179: 1-Hexadecyl-sn-glycero-3-phosphocholine; 204: 4-Hydroxycinnamic acid (L-phenylalanine methyl ester) amide; 37: alpha-ketoglutarate; 353: Taurolithocholic acid; 40: Apigenin 7-glucoside; 87: Glutaric acid; 317: N-Acetylmannosamine; 196: 3-Acetoxypyridine; 26: 3-Phenylpropanoic acid; 325: PC(16:0/16:0); 304: Myristoleic acid; 147: Phenyllactic acid.



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(Bi et al., 2022). The protective effect of Rg3 might be mediated by upregulation of mRNA expression of *mTOR, SOD-1*, and *HO-1* (Bi et al., 2022). In this study, using LC-MS profiling, 17 different metabolites were identified in the comparison Model vs. Control, which were related to oxidative stress, inflammation and degradation of protein and lipid. In addition, 18 different metabolites were identified in the comparison Rg3 vs. Model, which were involved in BCAA metabolism, apoptosis and mTOR signal pathway.

Metabolic analysis is an efficient and fast tool widely used in the research of disease diagnosis and drug targets (Shao et al., 2018). To the best of our knowledge, this is the first study to identify serum metabolites associated with the pathogenesis and the protection against stress in broilers. Previous studies demonstrated LPS challenge caused alterations in lipid and protein metabolism in different animals (Wan et al., 2017; Wang et al., 2017; Chirivi et al., 2022). In present study, taurolithocholic acid, myristoleic acid, stearoylcarnitine, PC (16:0/16:0), 1-Hexadecyl-sn-glycero-3-phosphocholine and Ser-Arg were significantly altered, suggesting that the abnormal degradation of lipid and protein were involved in the pathogenesis of stress in chicks (Ozawa et al., 2020; Heidenreich et al., 2021). Therefore, the changed metabolites may explain the decreased growth performance of broilers with stress induced by LPS challenge. Inflammatory response has been recognized as the major contributor to the pathogenesis of stress in broilers challenged with LPS (Liu et al., 2015). The inflammatory cytokines could promote glucocorticoid secretion and reduce the secretion of growth hormone through the neuro-endocrine system, resulting in decreased appetite and feed intake (Zhang et al., 2017). In addition, the proinflammatory cytokines accelerate glycogen hydrolysis and glucose production, as well as promote adipolysis and proteolysis, leading to reduced synthesis of protein and lipid (Li et al., 2017; Zhang et al., 2017). In the present study, increased phenyllactic acid and alpha-ketoglutarate, both of which have potential anti-inflammation effect are detected in Model birds (Asadi Shahmirzadi et al., 2020; Zhou et al., 2021). Production of proinflammatory cytokines and inducible proinflammatory enzymes such as iNOS release free radicals and induce oxidative stress, causing cell apoptosis and tissue damage, which is another explanation for growth inhibition (Han et al., 2020; Brenner et al., 2013). In this study, 3-phenylpropanoic acid, biliverdin and glutaric acid are related to the process of oxidative stress. 3-phenylpropanoic acid is a conjugate acid of 3-phenylpropionate which is a metabolite of phenylalanine obtained by deamination from phenylpropanoid pathway, while glutaric acid is a downstream oxidative metabolite of low density lipoprotein (**LDL**) in serum (Turlin et al., 2005; Ametaj et al., 2010; Vinaixa et al., 2011). Taken together, our results are consistent with the concept that inflammation and oxidative stress are involved in the development of LPS-induced stress in broiler chicks at biochemical and metabolism level. Moreover, these serum metabolic abnormalities such as phenyllactic acid and 3-phenylpropanoic acid deserve to further verification as biomarkers of stress in broiler chicks.

While 59% of different metabolites in the comparison Control vs. Model were downregulated, 83% of

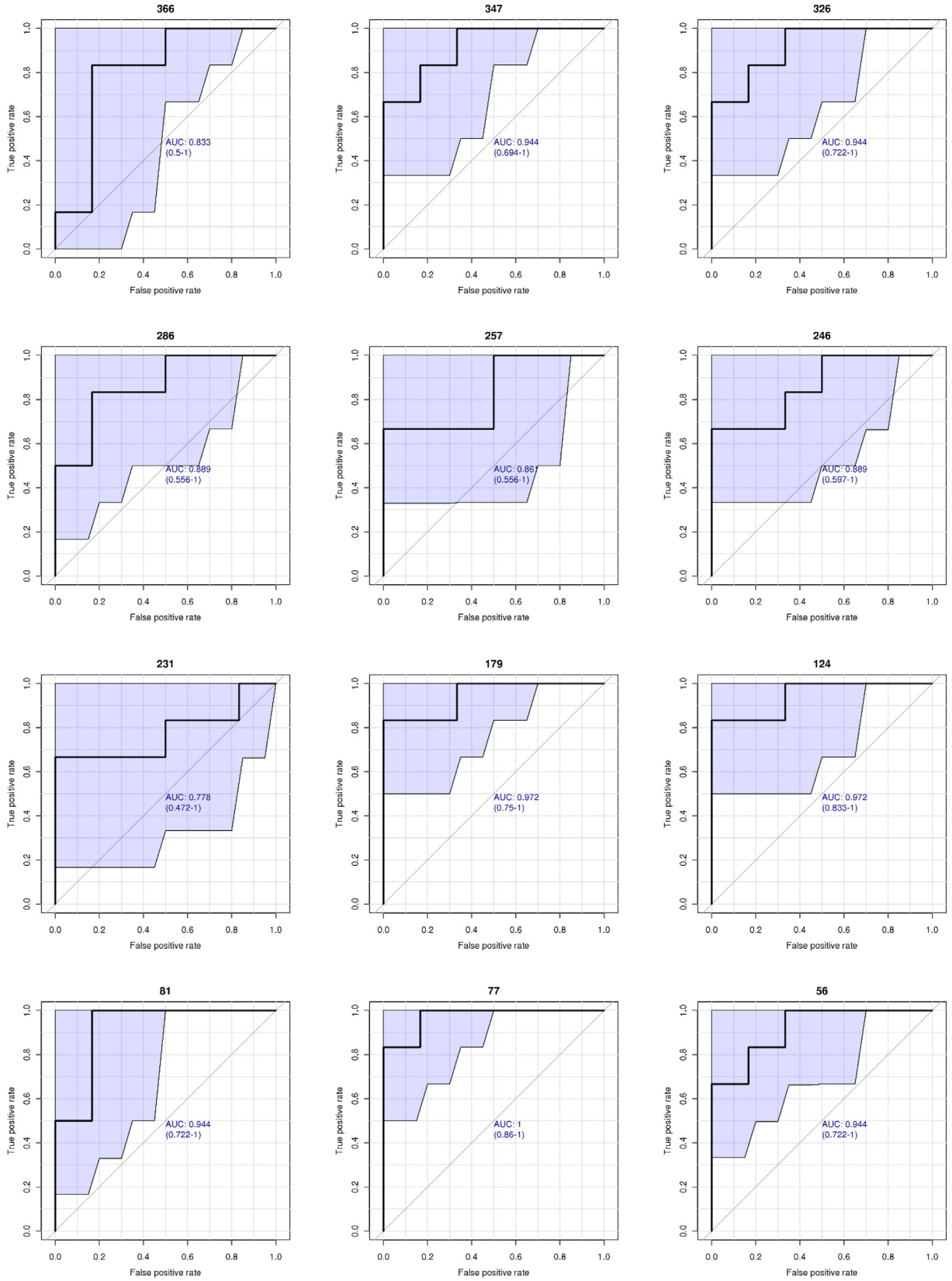
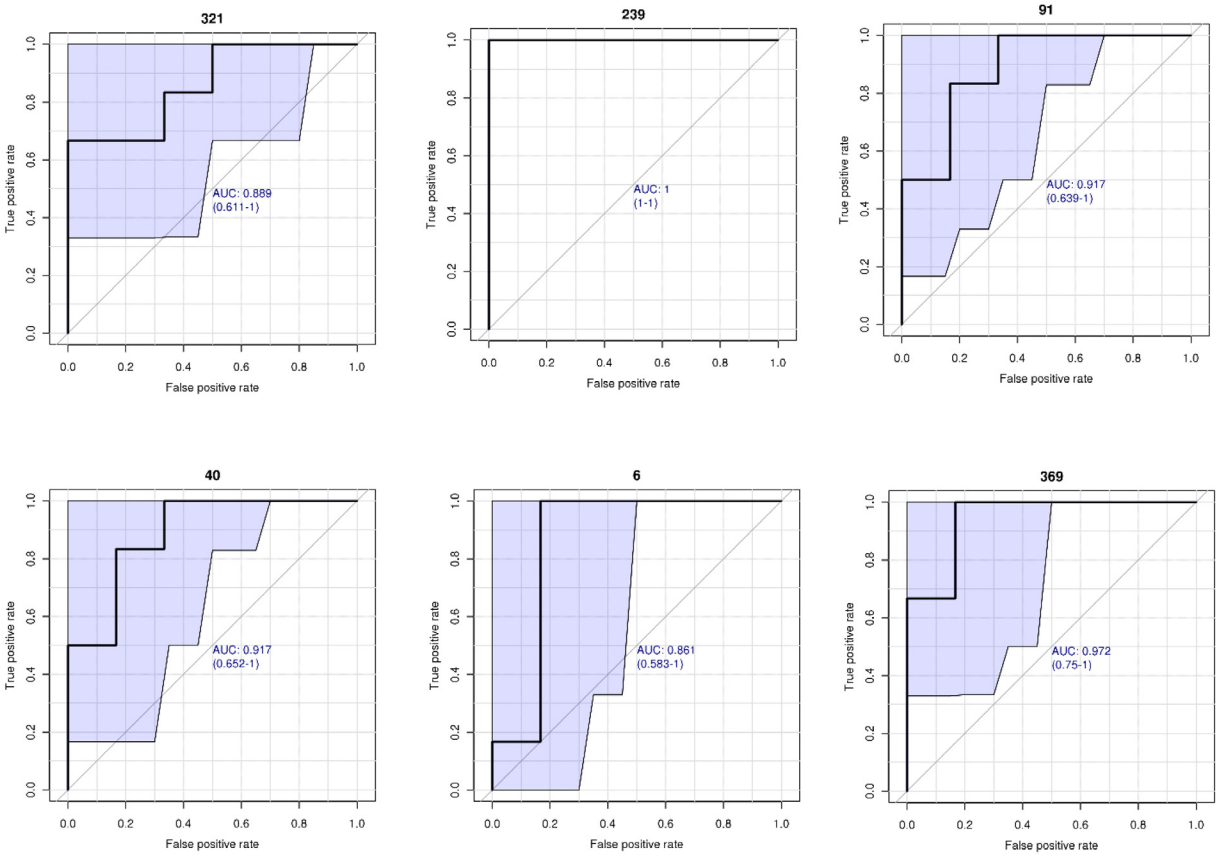


Figure 3. ROC curve analysis for metabolites in the comparison Rg3 vs. Model. 239: Cytosine; 124: L-Valine; 77: Enterostatin human; 179: 1-Hexadecyl-sn-glycero-3-phosphocholine; 326: Pentanol, 5-amino-; 347: Sphingosine; 56: Cytidine; 369: Val-Tyr; 40: Apigenin 7-glucoside; 91: Guanosine; 246: Diethanolamine; 231: Bisindolylmaleimide IV; 81: Gamma-Glutamyl cysteine; 286: L-Leucine; 257: Edaravone; 6: 1-Hexadecanoyl-sn-glycero-3-phosphoethanolamine; 321: Nicotinamide; 366: Uracil.



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discriminant metabolites in the comparison Rg3 vs. Model were upregulated. Notably, the levels of guanosine increased by 88% in the Rg3 group when compared with the Model broilers. Guanosine is a guanine-based purine released by astrocytes and protects cells against oxidative stress

by associating the heme oxygenase1 (**HO-1)** signaling pathway (Bellaver et al., 2015). Upregulation of HO-1 may inhibit activation of NF-κB and decrease production of pro-inflammatory cytokines (Quincozes-Santos et al., 2014). Furthermore, the downstream metabolites of HO-1, such as bilirubin and CO could promote generation of eNOS and inhibit iNOS induced NO production (Otterbein et al., 2000; Wang et al., 2004; Idelman et al., 2015). This is consistent with our previous observation on inflammatory response and oxidative stress (Bi et al., 2022). Leucine may act as a potential agent in reducing body temperature and confer thermotolerance in broiler chicks (Han et al., 2017). Interestingly, the decreased body temperature and reversed clinical expression associated with increased L-leucine was observed in Rg3 group. The alleviated oxidative damage in cells and tissues in turn reduced cell apoptosis evidenced by decreased sphingosine. As a messenger of apoptosis, the phosphorylation of sphingosine diminishes apoptosis, while dephosphorylation of sphingosine 1-phosphate potentiates it (Ospina-Rojas et al., 2017). To confirm the presume, a western blot on expression of sphingosine and phosphorylation of sphingosine is needed in a further study. Nevertheless, numerous studies have confirmed the effect of Rg3 on antioxidant enzymes and peroxidation of lipid and protein. Wei et al. (2012) found that injection of Rg3 significantly inhibited oxidative stress by elevating the capacity

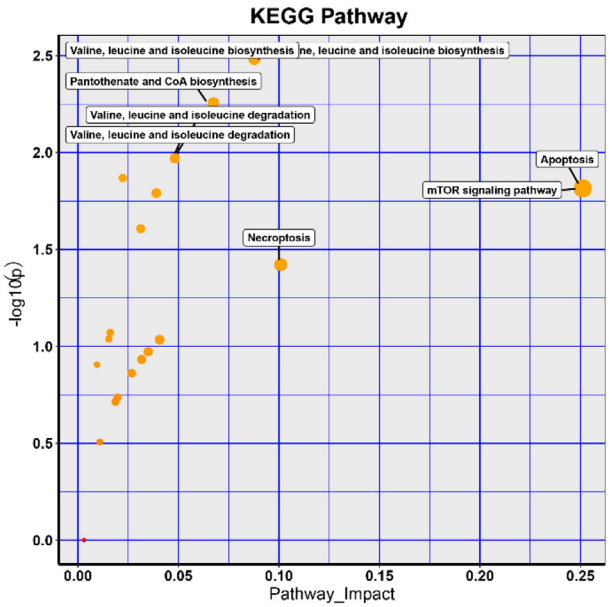

Figure 4. Metabolome view from pathway analysis performed using MetaboAnalyst. Metabolites of Rg3 vs. Model were used for KEGG pathways analysis. Pathways with impact value >0.1 or $-\log_{10}(P) > 1.5$ are labeled. $-\log_{10}(P)$ is from the original P-value calculated from the enrichment analysis. The x-axis represents the pathway impact, and y-axis represents the pathway enrichment. Larger sizes and darker colors represent higher pathway impact values and higher pathway enrichment.

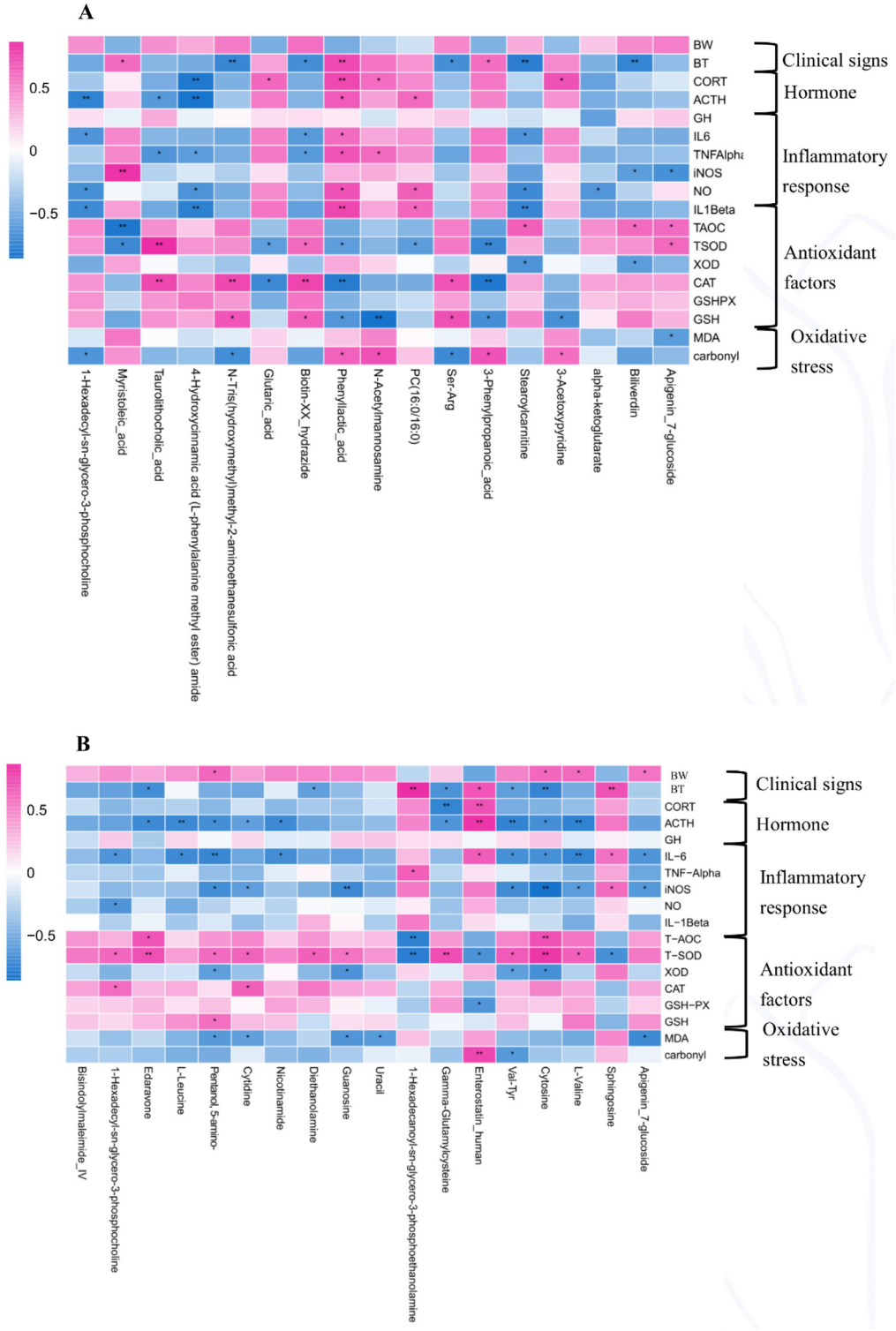

Figure 5. (A) Correlation analysis between biochemical indicators and potential biomarkers in the comparison Model vs. Control. (B) Correlation analysis between biochemical indicators and potential biomarkers in the comparison Rg3 vs. Model. Abbreviations: ACTH, adrenocorticotropic hormone; BW, body weight; BT, body temperature; CORT, corticosterone; GH, growth hormone.

of T-AOC, T-SOD and CAT, as well as decreasing the activity of XOD and the levels of MDA content. Li et al. (2020) demonstrated the antioxidant enzymes including CAT and SOD were elevated and the MDA content were decreased in mice in 20 (R)-Rg3-treated group with oxidative stress

In humans and animals, leucine, isoleucine, and valine are defined as branched chain amino acids (**BCAAs**) and play crucial roles in regulating energy homeostasis, nutrition metabolism, gut health, immunity, and disease (Nie et al., 2018). In chickens, a balanced supplement of leucine and valine are needed to optimize weight gain and feed conversion in low-CP diets (Ospina-Rojas et al., 2017). Conversely, low BCAAs levels suppress fatty acid synthesis and increase fatty acid β-oxidation (Bai et al., 2015). In this study, the upregulated L-valine and L-

leucine may partly explain the enhanced weight gain and decreased MDA in broiler chickens of Rg3 group. In the present study, KEGG pathway analysis revealed that metabolites were significantly involved in the BCAA metabolism and mTOR signaling pathway. Consistently, upregulating mRNA expression of mTOR in Rg3 group was demonstrated in liver of broiler chicks, suggesting that the protective effect of Rg3 on stress may be related to regulation of mTOR (Bi et al., 2022).

In conclusion, metabolomic analysis revealed potential serum metabolites involved in oxidative stress and inflammation, degradation of lipid and protein in broiler chicks with immune stress. In addition, the protective effect of Rg3 on the stressed chicks may be largely mediated by regulating mTOR, apoptosis and BCAA at the level of metabolism. These results suggested the potential biomarkers involved in the pathogenesis and prevention of stress. We also proposed that the differential metabolites such as biliverdin, alpha-ketoglutarate and leucine could be used in the control of immune stress in broiler chicks.

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Availability of data and materials: All data generated or analyzed during this study are included in this published article.

DISCLOSURES

The authors declare no competing financial interests.

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