Hepatic lipid metabolomics in response to heat stress in local broiler chickens breed (Huaixiang chickens)

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Abstract
High-temperature environment-induced heat stress (HS) is a hazard environmental element for animals, leading to dramatic changes in physiological and metabolic function. However, the metabolomic-level mechanisms underlying lipid metabolism in liver of slow-growing broilers are still obscure. The present study investigated the effects of HS on hepatic lipidomics in Chinese indigenous slow-growing broilers (Huaixiang chickens). The study includes two treatments, each treatment had 5 replicates with 4 broilers per cage, where a total of 40 eight-week-old female Huaixiang chickens (average initial body weight of 840.75 ± 20.79 g) were randomly divided into normal temperature (NT) and HS groups for 4 weeks, and the broilers of NT and HS groups were exposed to 21.3 ± 1.2°C and 32.5 ± 1.4°C respectively. The relative humidity of the two groups was maintained at 55%–70%. The liquid chromatography-mass spectrometry (LC-MS)-based metabolomics were conducted to evaluate the changes in hepatic lipidomics of broilers. The results showed that there were 12 differential metabolites between the two treatments. Compared with the NT group, HS group reduced the levels of hepatic phosphatidylcholine (PC) (16:0/16:0), PC (16:0/18:2), triglyceride (TG) (16:0/16:1/18:1), TG (18:0/18:1/20:4) (VIP > 1 and p < 0.05), while increased PC (18:1/20:3), PC (18:0/18:1), PC (18:1/18:1), PC (18:0/22:5), dimethylphosphatidyl ethanolamine (dMePE) (14:0/18:3), dMePE (18:0/18:1) and dMePE (16:0/20:3) levels (Variable Importance in the Projection; VIP > 1 and p < 0.05). In addition, according to the analysis of metabolic pathway, the pathways of linoleic acid, alpha-linolenic acid, glycerolipid and glycerophospholipid metabolism were involved in the effects of HS on hepatic lipid metabolism of broilers (p < 0.05). In conclusion, HS altered the hepatic lipid metabolism mainly through linoleic acid, alpha-linolenic acid, glycerolipid and glycerophospholipid metabolism involved in the effects of HS on hepatic lipid metabolism of broilers (p < 0.05). In conclusion, HS altered the hepatic lipid metabolism mainly through linoleic acid, alpha-linolenic acid, glycerolipid and glycerophospholipid metabolism pathway in indigenous broilers. These findings provided novel insights into the role of HS on hepatic lipidomics in Chinese indigenous broiler chickens.

KEYWORDS
heat stress, indigenous broilers, lipid metabolomics, liver
1 | INTRODUCTION

Under economic stimulus, the high stocking density causes HS, which can adversely affect the health and production of animals, especially in summer (Lolli et al., 2010; Quinteiro-Filho et al., 2010). Broilers are particularly sensitive to HS (Mascarenhas et al., 2018; Sakamoto et al., 2020). Therefore, HS has been considered to be one of the main environmental factors affecting broilers' production. HS can reduce feed intake, slow down the growth rate and lead to systemic immune disorders in broilers (Goo et al., 2019; Liu et al., 2019). In addition, HS decreased the basal metabolic rate and significantly changed the level of fat metabolism in broilers, resulting in excessive fat deposition (Sato et al., 2006; Shim et al., 2016). The liver is one of the important lipid metabolic organs in broilers, which has crucial functions such as secretion of bile, storage of hepatic glycogen and detoxification (Yarru et al., 2009). Lipid metabolism is closely related to the maintenance of dynamic energy balance and the physiological function of broilers (He et al., 2018). When broilers under HS, the energy balance in the body is destroyed, and too much energy is used for fat storage, thus damaging hepatic tissue and function (Lu et al., 2019). Previous studies in the liver of broilers have shown that HS altered the expression and the activity of various enzymes and played vital role in the regulation of lipid metabolism (Flees et al., 2017; Tang et al., 2015). Meanwhile, HS induced changes in certain hepatic parameters and lipid metabolites in broilers, indicating that hepatic lipid metabolites are sensitive and valuable parameters for lipid metabolism research in broilers exposed to HS (Shim et al., 2006). In this regard, Lu et al. (2019) demonstrated that HS increases the level of hepatic triglycerides (TG), total cholesterol (TCHO) and fatty acid synthase (FAS) in Arbor Acres (AA) broilers.

In recent years, metabolomics has become the leading means of systems biology research, and has been widely used in biomedical, pharmaceutical and toxicological research. Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) have been extensively used in metabolic profiling analysis of various samples due to its high resolution and detection sensitivity (Bujak et al., 2015; Li et al., 2018). According to earlier reports, it has been suggested that the mechanism of lipid metabolism regulation and the specific lipid metabolites can be researched by lipidomics analysis in various life phenomena (Cai et al., 2011). Lipids have many important biological functions, and abnormal lipid metabolism can cause many animal diseases (Hertz et al., 2008). The identification and comprehensive analysis of lipid metabolites in organisms at the lipidomic level can reveal the changes in lipid metabolism and related regulatory mechanisms under internal or external stimulation (Shevchenko & Simons, 2010). However, there are limited reports on the effects of HS on hepatic lipid metabolism in broilers based at lipidomic level, especially for the indigenous slow-growing broilers.

Indigenous yellow-feathered broilers are gradually favoured by consumers because of the excellent meat quality. Huaixiang chicken is a famous Chinese indigenous yellow-feathered slow-growing broiler breed. It has the advantages of rough feeding tolerance, good foraging ability and strong disease resistance, which is widely raised in South China (Guo et al., 2021; Liu et al., 2019; Shi et al., 2016). In addition, this breed of chicken has the advantages of tender meat and low content of fat. The high environmental temperature in South China often causes HS in indigenous slow-growing broilers, which may affect their lipid metabolism and leads to excessive fat deposition, thus negatively influencing the carcass traits, meat quality and health status. However, few studies have focused on the effects of HS on hepatic lipid metabolomics in Huaixiang chicken. Hence, this study attempted to analyse the characteristics of lipid metabolism in the liver of Huaixiang chickens under HS through lipid metabolomics study, and to lay a foundation for the screening of lipid biomarkers in indigenous broilers under HS. It also expanded understandings of the effects of HS on hepatic lipidomics in Chinese indigenous yellow-feathered broiler chickens.

2 | MATERIALS AND METHODS

2.1 | Animals, diet and experimental procedures

A total of 40 eight-week-old female Huaixiang chickens (average initial body weight of 840.75 ± 20.79 g) were randomly divided into two treatments that included normal temperature (NT) and heat stress (HS) groups for 4 weeks. Each treatment had 5 replicates with 4 broilers per cage, and there was no significant difference in initial body weight between repetitions and treatments. The broilers of NT and HS groups were exposed to 21.3 ± 1.2°C and 32.5 ± 1.4°C, respectively, from 9 a.m. to 5 p.m., which lasted for 8 hr every day. The relative humidity of the two groups was maintained at 55%-70% during the whole experimental period. All the birds were raised in the chicken house with environmental control equipment, the temperature and humidity are controlled by equipment. The temperature and humidity data were recorded at 6.30 a.m., 9 a.m., 12 a.m., 2 p.m., 5 p.m., 8 p.m. and 11 p.m. every day from six different locations in the chicken house. The cage size was 90 (length) x 70 (width) x 40 (height) cm. All birds were given ad libitum access to feed and water. During the experimental period, all broilers were fed with the diets (Table 1) based on corn–soybean meal and the basal diet formula was based on Chinese Chicken Feeding Standard (NY/T 33-2004) (MAPRC, 2004).

2.2 | Sampling and preparation

At the end of the trial, five birds (one bird from each repeat) were randomly selected from each treatment and killed by cervical dislocation. Subsequently, the liver samples were collected immediately, then the liver samples were immersed in liquid nitrogen and stored at −80°C for further determination and analysis (Guo et al., 2020; Liu et al., 2020).

For lipid extraction, chloroform/methanol solution was added to each sample. The solution was mixed by a vortex mixer for 5 min. Then, the mixture was centrifuged at 8000 g for 10 min at 4°C. The supernatant was put into the clean test tube, and the precipitates
were extracted twice with 2 ml chloroform/methanol solution. All the supernatants were dried by N2 and then dissolved with chloroform/methanol (2:1, v:v). The supernatant (200 μl) was transferred to vials for detection. Dionex UltiMate 3000 (UHPLC)-Thermo Orbitrap Elite was used for liquid LC-MS analysis.

### TABLE 1 Basal diet composition (as-fed basis)a

| Item            | Contents (%) |
|-----------------|-------------|
| Ingredients     |             |
| Corn            | 67.00       |
| Soybean meal    | 23.00       |
| Wheat bran      | 4.00        |
| Fish meal       | 3.00        |
| Limestone       | 1.50        |
| CaHPO<sub>4</sub> | 1.00        |
| Premix<sup>b</sup> | 0.50      |
| Total           | 100.00      |

Nutrient levels<sup>c</sup>

| Item | Contents (MJ/kg) |
|------|------------------|
| ME   | 11.94            |
| Ca   | 18.22            |
| Cr   | 0.98             |
| Met  | 0.32             |
| Cysteine | 0.31    |
| Lys  | 0.90             |
| total phosphorus | 0.51 |

<sup>a</sup>The basal diet formula was based on the Chinese Chicken Feeding Standard (NY/T 33-2004) (MAPRC, 2004).

<sup>b</sup>Premix provided per kilogram of diet: 5,000 IU of vitamin A, 1,000 IU of vitamin E, 0.5 mg of vitamin K<sub>3</sub>, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B<sub>6</sub>, 10 μg of vitamin B<sub>12</sub>, 25 mg of niacin, 0.55 mg of folic acid, 0.2 mg of biotin, 500 mg of choline, and 10.5 mg of pantothenic acid. 60 mg of Zn, 80 mg of Mn, 80 mg of Fe, 3.75 mg of Cu and 0.35 mg of I.

<sup>c</sup>Except for metabolizable energy (ME) (Lopez & Leeson, 2005), others are measured values.

### 2.3 | LC-MS analysis

The LC-MS analysis was done as described previously (Vinayavekhin et al., 2016). The chromatographic column used was Waters UPLC<sup>®</sup>BEH C18 (1.7 μm 100*2.1 mm). Mass spectrometry was operated in both positive and negative ion modes. Mobile phase: A: aqueous solution with 0.1% formic acid (0.1% 1 mmol/L NH₄COOH), B: acetonitrile/isopropanol solution (the ratio of acetonitrile and isopropanol was 1:1, 0.1% 1 mmol/L NH₄COON, 0.1% HCOOH). Flow rate: 0.40 ml/min. Column temperature: 45°C. Injection volume: 4 μl. Gradient elution conditions were as follows: 0–2 min, 35%–80% B; 2–9 min, 80%–100% B; 9–16 min, 100% B; 16–20 min and 100%–35% B. Post-time was set as 3 min for system balance.

Mass spectrometry was operated in both positive and negative ion modes. The parameters optimized were as follows. Positive mode, Heater Temp 300°C; Sheath Gas Flow rate 45 arb; Aux Gas Flow Rate 15 arb; Sweep Gas Flow Rate 1 arb; spray voltage 3.0 KV; Capillary Temp 350°C, S-Lens RF Level 30% and Mass range: m/z 200–1,500. Negative mode, Heater Temp 300°C; Sheath Gas Flow rate 45 arb; Aux Gas Flow Rate 15 arb; Sweep Gas Flow Rate 1 arb; spray voltage 2.5 KV; Capillary Temp 350°C, S-Lens RF Level 60% and Mass range: m/z 200–1,500.

### 2.4 | Metabolomic data analysis

Raw data were converted the common (mz.data) format by Agilent MassHunter Qualitative Analysis B.08.00 software (Agilent Technologies, USA). In the R software platform, the XCMS program was used in peak identification, retention time correction and automatic integration pre-treatment. Then, the data were subjected to internal standard normalization (Zhao et al., 2019). After editing, the data matrices were import into SIMCA-P 13.0 (Umetrics, Umea, Sweden), mean centred and scaled to Pareto variance. Then, multivariate analysis was conducted. Data were analysed by partial least-square discriminant analysis (PLS-DA), and orthogonal projections to latent structures discriminant analysis (OPLS-DA). Differential metabolites were screened out by Variable Importance in the Projection (VIP) value of OPLS-DA model (VIP ≥ 1) and independent sample t test (p < 0.05).

The differential metabolites of HS and NT groups were mapped to the Kyoto Encyclopedia of Genes and Genomes Identifier (KEGG ID) by online software MetaboAnalyst. A pathway analysis was implemented. House mouse (Mus musculus) was selected as a model organism. The significant pathways (p < 0.05) were selected using KEGG.

### 3 | RESULTS

#### 3.1 | PLS-DA and OPLS-DA analysis

The PLS-DA scores plots are depicted in Figure 1. There was an obvious separating trend between the groups. In the model of positive mode of HS and NT group, R²X = 0.713, R²Y = 0.670 and Q² = 0.142. In the model of negative mode of HS and NT group, R²X = 0.650, R²Y = 0.957 and Q² = 0.752. OPLS-DA scores plots are drawn in Figure 2. There was an obvious separating trend between the groups. In the model of positive mode of HS and NT group, R²X = 0.713, R²Y = 0.670 and Q² = 0.518. In the model of negative mode of HS and NT groups, R²X = 0.650, R²Y = 0.957 and Q² = 0.323.

#### 3.2 | Metabolites analysis

According to OPLS-DA model (VIP ≥ 1) and independent sample t test (p < 0.05), Tables 2 and 3 showed that 12 differential metabolites were identified. Compared with the NT group, the concentrations of phosphatidylcholine (PC) (16:0/16:0), PC (16:0/18:2), triglyceride...
(TG) (16:0/16:1/18:1) and TG (18:0/18:1/20:4) were lower (VIP > 1 and p < 0.05), while PC (18:1/20:3), PC (18:0/18:1), PC (18:1/18:1), PC (18:0/22:5), dimethylphosphatidylethanolamine (dMePE) (14:0/18:3), dMePE (18:0/18:1) and dMePE (16:0/20:3) was higher (VIP > 1 and p < 0.05) in the HS group.

3.3 | Analysis of related metabolic pathways

As indicated in Table 4, pathway analysis showed that HS changed (p < 0.05) the pathway of alpha-Linolenic acid (Figure 3), glycerolipid (Figure 4) and glycerophospholipid metabolism (Figure 5) in comparison with NT group. Notably, HS had extremely significant effect on linoleic acid metabolic pathway (p < 0.01) (Figure 6), and an influencing trend was observed in the pathway of arachidonic acid metabolism (p = 0.0502).

4 | DISCUSSION

Ambient temperature is one of the most important factors that affects poultry production in the tropics and subtropics (Chauhan & Ghosh, 2014; Mohamed et al., 2019). In recent years, many previous studies have been done on the harmful effects caused by HS in broilers (De Antonio et al., 2017; Liu et al., 2019). The deleterious impacts on productivity and multiple physiological functions of broilers by HS, including the decline of growth rate, the disturbance of lipid metabolism and the change of carcass composition, which results in huge economic losses to broilers industry (Belhadj Slimen et al., 2016; Lu et al., 2007; Moraes et al., 2003). Broilers exposed to the HS showed excessive fat deposition, which indicated that the process of lipid synthesis and metabolism has been changed in broilers (Ryder et al., 2004). The metabolic process of lipids in the body mainly includes the metabolism of triglycerides, phospholipids, cholesterol and plasma lipoproteins (Zeng et al., 2017). Previous studies have shown that HS inhibited hepatocyte proliferation, promoted hepatocyte apoptosis and induced hepatocyte necrosis in mice (Li et al., 2012; Thompson et al., 2014). It has also suggested that the hepatocytes of broilers exposed to HS showed “steatosis” (Lu et al., 2019). Lipid metabolism is closely related to hepatic function, and abnormal metabolic pathway may be related to the effects of HS on hepatic function (Lv et al., 2018). However, there are few reports about the effects of HS on hepatic lipidomics in indigenous slow-growing broiler chickens. Therefore, the study of hepatic lipidomics in broilers under HS is helpful to clarify the mechanism of the effects of HS on lipid metabolism in broilers under HS. In the present study, LC-MS was used to analyse the hepatic lipid metabolites of indigenous broilers. The results showed that HS altered the level of hepatic lipid metabolites, including TG, PC and dMePE. In addition, the identified differential metabolites were analysed by KEGG, and it showed that the different metabolites were mainly concentrated in the metabolic pathways of linoleic acid, α-linoleic acid, glycerol and glycerol phospholipid in the liver of broilers, and the effect on linoleic acid metabolic pathway was the most significant. Linoleic acid exists mainly in the form of glycerol ester in poultry (Kostogrys & Pisulewski, 2010). These results suggested that HS changed the hepatic lipid metabolic process in indigenous broilers.

Specifically, the result of this study showed that the hepatic TG content was decreased by chronic HS (4 weeks). As we all know, TG is one of the components of lipids, and the main function of TG is to supply and store energy, which can fix and protect internal organs of animals (Das et al., 2014). In vivo, TG synthesized are mainly in the liver, followed by adipose tissue (Almeida et al., 2013). It has been reported that HS broilers have reduced feed intake, resulting in energy deficiency, which in turn leads to lipid metabolism disorders (Cornejo et al., 2007). Therefore, the decrease in TG content may be due to the decrease in feed intake and the damage of hepatic function caused by HS. The low level of TG may also be due to species of broilers, diet

![Figure 1](image-url)
type and the growth stages. In addition, Zhou et al. (2018) found that long-term (72 h) HS significantly affected hepatic lipid metabolism in broilers, while short-term (24 h) HS had limited effect on hepatic lipid metabolism in broilers (Zhou et al., 2018). It is suggested that the TG level in liver is related to the duration of HS, however, the specific mechanism needs to be further studied.

In addition, lipids are the main substances in cells and have a variety of biological functions, including the construction of biofilms, the regulation of energy conversion and the transmission of cell signals (Ottaviani et al., 2011). Therefore, they are involved in a variety of biological processes, such as cell growth and differentiation. The phospholipid is an important part of biomembrane and the main lipid carrier in blood, which plays a pivotal role in activating cells and maintaining metabolism (Wang et al., 2003).

Moreover, phospholipids can promote fat metabolism, prevent fatty liver and reduce serum cholesterol (Li et al., 2010). As the main sources of phospholipids, PC and PE have crucial functions on the integrity of cell and organelle membranes (Luo et al., 2018). Also, it has been reported that dMePE was formed by methylation of phosphatidylethanolamine (PE) by S-adenosylmethionine, and the upregulation of dMePE level may be related to the increase in methylase activity. Li et al. (2012) found that HS destroys the function of hepatic cell membrane, which affects the value-added ability of cells. In addition, previous studies have shown that changes in the levels of PC and PE are related to steatohepatitis (Li et al., 2006). Therefore, it can be inferred that the damage of HS

**FIGURE 2** Score plot of orthogonal projections to latent structures discriminant analyses (OPLS-DA) derived from LC-MS profiles of hepatic samples obtained from HS group and NT group. (a) Positive mode (pos) and (b) negative mode (neg). (Blue) NT group and (Green) HS group

**TABLE 2** Differential metabolites in liver of broilers in the NT and HS group (pos)

| Lipid Ion          | FC (HS/NT) | VIP (HS&NT) | P-value | Change |
|-------------------|------------|-------------|---------|--------|
| PC                |            |             |         |        |
| (16:0/16:0)       | 0.0991     | 5.1779      | 0.0325  | ↓      |
| (16:0/18:2)       | 0.0602     | 2.7908      | 0.0163  | ↓      |
| (18:1/20:3)       | 3.8352     | 4.9310      | 0.0265  | ↑      |
| TG                |            |             |         |        |
| (16:0/16:0/18:1)  | 0.3422     | 1.3739      | 0.0140  | ↓      |
| (16:0/16:1/18:1)  | 0.2903     | 1.4507      | 0.0038  | ↓      |
| (18:0/18:1/20:4)  | 0.2113     | 2.4200      | 0.0097  | ↓      |

Note: NT, normal temperature group (21.3 ± 1.2°C); HS, heat stress group (32.5 ± 1.4°C). Phosphatidylcholine (PC); Triglyceride (TG). VIP, variable importance in the projection; FC, ratio of mean peak area of the HS group to the mean peak area of the NT group; ↑, metabolites with higher concentrations in the HS group than in the NT group with FC > 1; ↓, metabolites with lower concentrations in the HS group than in the NT group with FC < 1.

**TABLE 3** Differential metabolites in liver of broilers in the NT and HS group (neg)

| Lipid Ion          | FC (HS/NT) | VIP (HS&NT) | P-value | Change |
|-------------------|------------|-------------|---------|--------|
| PC                |            |             |         |        |
| (18:0/18:1)       | 2.8536     | 9.8355      | 0.0321  | ↑      |
| (18:1/18:1)       | 1.6958     | 8.5464      | 0.0143  | ↑      |
| (18:0/22:5)       | 2.2195     | 1.2616      | 0.0227  | ↑      |
| dMePE             |            |             |         |        |
| (14:0/18:3)       | 1.7515     | 1.1556      | 0.0303  | ↑      |
| (18:0/18:1)       | 1.4885     | 2.0134      | 0.0315  | ↑      |
| (16:0/20:3)       | 2.6628     | 1.7160      | 0.0323  | ↑      |

Note: NT, normal temperature group (21.3 ± 1.2°C); HS, heat stress group (32.5 ± 1.4°C). Phosphatidylcholine (PC); dimethylphosphatidylethanolamine (dMePE). VIP, variable importance in the projection; FC, ratio of mean peak area of the HS group to the mean peak area of the NT group; ↑, metabolites with higher concentrations in the HS group than in the NT group with FC > 1; ↓, metabolites with lower concentrations in the HS group than in the NT group with FC < 1.
to cell membrane and hepatic function may be due to the changes in hepatic PC and dMePE levels. Regarding the mechanism of the effects of HS on the levels of hepatic phospholipids, it has been suggested that HS can increase the activity of phospholipase A2 (PLA2) and promote the decomposition of phospholipids, thereby causing damage to cell membranes (Sahebkar, 2013). Therefore, the development of PLA2 inhibitors in future researches may help to ameliorate HS-induced impairment of phospholipids metabolism.

**TABLE 4** Analysis of related metabolic pathways

| Pathway name                        | P-value  |
|-------------------------------------|----------|
| Linoleic acid metabolism            | 0.0084   |
| alpha-Linolenic acid metabolism     | 0.0127   |
| Glycerolipid metabolism            | 0.0253   |
| Glycerophospholipid metabolism      | 0.0419   |
| Arachidonic acid metabolism         | 0.0502   |

Note: HS group versus NT group. p < 0.05 was considered to be statistically significant, 0.05 ≤ p < 0.10 was considered to be a tendency.

**FIGURE 3** Schematic overview of alpha-Linolenic acid metabolic pathway and some related metabolites in heat-stressed broilers. Red, metabolites in HS group versus NT group upregulation and cell membrane function in broilers. On the other hand, previous studies have shown that the chemical activators (phenformin and AICAR) of adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) inhibit both PC biosynthetic pathways in isolated hepatocytes (Kasturi et al., 2006). Also, it has been indicated that HS activates the activity of AMPK in piglet hepatocytes and reduces the synthesis of phospholipids, TG and cholesterol in hepatocytes (Zhang et al., 2007). Simultaneously, acetyl-CoA carboxylase (ACC) is a key enzyme in fatty acid synthesis, while AMPK can inhibit the activity of ACC through phosphorylation. According to previous reports, HS motivates the AMPK signalling pathway and inactivates ACC, thus interfering with the synthesis of fatty acid (Shen et al., 2019). Therefore, HS altered the hepatic PC, PE and dMePE metabolic levels might be involved in activation of AMPK signalling pathway during the broilers under a negative energy balance status (Zhou et al., 2018). It is suggested that improving dietary energy density and application of AMPK activity inhibiting additives may protect the cell membrane function and reduce the adverse effects of HS on hepatic lipid metabolism in indigenous broilers, this is worthy of further verification.
**FIGURE 4** Schematic overview of glycerolipid metabolic pathway and some related metabolites in heat-stressed broilers. Blue, metabolites in HS group versus NT group downregulation

**FIGURE 5** Schematic overview of glycerophospholipid metabolic pathway and some related metabolites in heat-stressed broilers. Red, metabolites in HS group versus NT group upregulation
In conclusion, HS altered the level of 12 hepatic lipid metabolites and affected hepatic linoleic acid, alpha-linolenic acid and glycerolipid, and glycerophospholipid metabolic pathways in indigenous broilers. This may be the mechanism of lipid metabolism that HS-induced hepatic dysfunction. The current findings provided new insights into the effects of HS on lipid metabolism at metabolomic level in indigenous slow-growing broilers.

ETHICS APPROVAL
The present study was carried out at the College of Coastal Agricultural Sciences, Guangdong Ocean University, Zhanjiang, China. The experimental protocol used in the study was approved by the Animal Care Committee of Guangdong Ocean University, Zhanjiang, China.

CONFLICTS OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
Wen-Chao Liu: Conceptualization; Wen-Chao Liu & Yan Guo: Methodology; Yan Guo, Jia-Hao Liao & Zi-Long Liang: Formal analysis; Yan Guo, Jia-Hao Liao & Zi-Long Liang: Data curation; Yan Guo: Writing-original draft preparation; Wen-Chao Liu & Balamuralikrishnan Balasubramanian: Writing-review and editing; Wen-Chao Liu: Supervision; Wen-Chao Liu: Project administration; Wen-Chao Liu: Funding acquisition.

DATA AVAILABILITY STATEMENT
All public data generated or analysed during this study are included in the study.

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