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Effect of heat stress on ileal microbial community of indigenous yellow-feather broilers based on 16S rRNA gene sequencing

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Abstract

Objectives: The broiler chickens are susceptible to heat stress (HS), including the indigenous broilers raised in tropical and subtropical regions. HS caused intestinal dysfunction and disrupted the gut microbiota. However, the researches about the effects of HS on ileal microbiome of indigenous broilers are limited. Therefore, this experiment used 16S rRNA sequencing to analyse the ileal microbial community in indigenous yellow-feather broilers under HS.

Material and methods: The single factor completely random design was used in the present study, and forty 8-week-old Chinese indigenous yellow-feather broilers (Huaixiang chickens) were randomly divided into two treatments: normal temperature (NT) group and HS group. There are five replications with four broilers per replicate in each group. The broilers in NT group were raised at 21.3 ± 1.2°C during the whole experimental period, the broilers in HS group were exposed to 32.5 ± 1.4°C for 8 h/day from 9:00 am to 17:00 pm and the temperature of rest time is consistent with NT group. The experiment lasted for 4 weeks.

Results: The results showed that HS exposure had no significant effects on the alpha diversity index of ileal microflora of broilers, including the Shannon, Simpson, Chao1 and ACE indexes ($p > 0.05$). At the genus level, HS significantly reduced the relative abundance of *Campylobacter* ($p < 0.05$), and increased the abundance of *Delftia* ($p < 0.05$). In addition, prediction of microbial community function indicated that HS significantly enhanced the abundance of the microflora related to lipid metabolism, carbohydrate metabolism and xenobiotics biodegradation and metabolism and reduced the abundance of the microflora related to nucleotide metabolism and amino acid metabolism.

Conclusions: Taken together, the present study revealed that chronic HS (4 weeks) exposure changes the abundance of the ileal microflora of broilers. These findings

provided new insights into the role of HS in influencing ileal microbial community in indigenous broilers.

KEYWORDS
16S rRNA sequencing, heat stress, ileal microbial community, yellow-feather broilers

1 | INTRODUCTION

Broiler chickens suffer from various stress conditions, including the environmental stressors, which negatively influence the broilers' health and productivity (Liu et al., 2021a; Najafi et al., 2015; Zhu et al., 2019). The broilers are highly sensitive to high-temperature environment due to the feathers and high productivity. The optimal environment temperature range for broiler growth is between 21 and 26°C. When the ambient temperature exceeds 32°C, it will induce heat stress (HS) in broilers (Liu et al., 2019). According to the duration of HS, it can be divided into acute HS and chronic HS (Liu et al., 2021c; Zhu, 2020). Acute HS refers to the short and rapid increase of the ambient temperature and the chronic HS refers to the relative long HS period (≥3 days) (Zhu, 2020). However, with the global warming, especially in tropical and subtropical regions, chronic HS in summer has become one of the biggest challenges in broiler production (Chauhan et al., 2021; Liu et al., 2021b). It has been suggested that chronic HS exposure causes impairment of physiological functions and organ health, such as endocrine disorders and intestinal injury of broilers (Emami et al., 2021; Hirakawa et al., 2020; Liu et al., 2021c). Along with the multiple physiological and organ disturbances, HS also results in increased mortality and reduced growth performance, which consequently leads to significant economic losses for broilers producers (Quinteiro-Filho et al., 2010). Therefore, the studies that explore the effects of HS on broilers are beneficial to advocate an effective strategy to protect broiler chickens under HS conditions.

The intestinal function has significant impacts on the health and growth performance of poultry (Kadykalo et al., 2018; Kaldhudal et al., 2016; Skinner et al., 2010). Also, the intestine is an important barrier to maintain the stable internal environment (Guo et al., 2020; Liu et al., 2020; Pan & Yu 2014). Emerging researches have demonstrated that the gut microbiota has multiple biological functions and plays critical roles during HS in broilers (Liu et al., 2021a; Meng et al., 2018; Mohamed et al., 2018). It has been suggested that the gut microbiota could protect the host from pathogens and enhance host immunity (Hudak et al., 2017; Roundjl et al., 2011; Serino et al., 2012; Swartztd et al., 2013). Therefore, the balance of gut microbiota is of great significance to the broilers' health and growth performance (Thomas et al., 2019). Recently, omics technology has become an important method to study the gut and/or organs health and development of broilers (Zhao et al., 2021). Among the omics technologies, 16S rRNA sequencing technology has its OUT standing ability to identify microorganisms with the rapid expansion of the 16S rRNA database, which has been widely used in the detection of intestinal microbiota (Yoshimura et al., 2011; Zhao & Chen, 2015). In this regard, She et al. (2018) reported that the application of antibiotics (chlortetracycline) changed the content of specific bacterial in the ileum and cecum of AA broilers via 16S rRNA high-throughput sequence analyses. Shi et al. (2019) used 16S rRNA technology to study the effects of HS on the cecum microflora structure in Lingnan yellow broilers, and they observed that the HS broilers had more bacteria and cyanobacteria than the normal temperature group at phylum level.

As high meat quality indigenous yellow-feather broiler breed, Huaixiang chickens, are widely farmed in southern China (Guo et al., 2021a, b, c). However, the high ambient temperature in southern China always causes HS in production of Huaixiang chickens, thus resulting in a decline in intestinal health, and ultimately negatively affects the productivity (Liu et al., 2019; Liu et al., 2021a, b). Because HS results in insufficient feed intake and intestinal physical barrier damage, so the balance of gut microbiota is easily disturbed by HS (Liang et al., 2021; Richards et al., 2010; Rostagno et al., 2020). The ileum is the mid-hindgut in poultry which is an important site for digestion and nutrient absorption and has rich microorganisms (Shaufi et al., 2015). Nevertheless, there are no reports on the effects of HS on the ileal microbiota of indigenous yellow-feather broilers based on 16S rRNA sequencing. Therefore, this study was conducted to evaluate the effects of HS on ileal microbial community in yellow-feather broilers (Huaixiang chickens) via 16S rRNA high-throughput sequence analyses.

2 | MATERIALS AND METHODS

2.1 | Experimental animals and their feeding and management

A total of 40 female yellow-feather broilers (Huaixing chickens, 8-week-old) with an average weight of 840.75 ± 20.79 g were randomly divided into two treatments: NT and HS group. There were five replicates in each group and four chickens in each replicate. This experiment adopted the method which was a single factor completely random design. Broilers in NT group were kept at an ambient temperature of 21.3 ± 1.2°C throughout the experimental period. Broilers in HS group were exposed to a high temperature of 32.5 ± 1.4°C (8 h/day, from 9:00 am to 17:00 pm). The relative humidity of NT and HS was maintained at 55–70%. In order to better control the environment (heating is easier in practice), the present animal trail has been carried out in winter from mid-December to mid-January. The temperature of the two groups is controlled by heating equipment to reach the experimentally set

TABLE 1 Basal diet composition

Item	Contents (%)
Ingredients	
Corn	67.00
Soybean meal	23.00
Wheat bran	4.00
Fish meal	3.00
Limestone	1.50
$CaHPO_4$	1.00
Premix[1]	0.50
Total	100.00
Nutrient levels[2]	
ME (MJ/kg)	11.94
Crude protein (%)	18.22
Ca (%)	0.98
Met (%)	0.32
Cystine (%)	0.31
Lys (%)	0.90
Total phosphorus (%)	0.51

[1]Premix provided per kilogram of diet: 5000 IU of vitamin A, 1000 IU of vitamin D3, 10 IU of vitamin E, 0.5 mg of vitamin K3, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B6, 10 μg of vitamin B12, 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 and 60 mg of Zn, 80 mg of Mn, 80 mg of Fe, 3.75 mg of Cu and 0.35 mg of I.
[2]Except for metabolisable energy (ME), others were measured values.

temperature, and simulated HS status in summer. The feeding trail lasted 4 weeks. During the experiment, chickens were provided with cages of 90 (length) × 70 (width) × 40 (height) cm, and it was ensured that all chickens could freely obtain water and feed. All broilers were fed corn-soybean meal basal diet (Table 1), and the diet formula according to the Chinese chicken breeding standard (NY/T33-2004).

2.2 | Sample collection

After fasting for 12 h, one bird was randomly selected from each replicate and slaughtered by jugular vein bloodletting; 5% of the benzalkonium bromide soaked for 5 min. Subsequently, the intestinal tract was separated and opened longitudinally, and about 2 g of ileum contents were collected in EP tube and put into the liquid nitrogen for prefrozen, and then transferred to the refrigerator at −80°C for subsequent microbiological analysis.

2.3 | DNA extraction and 16S rRNA sequencing of ileal microflora

Personalibio Bio was commissioned to use Illumina MiSeq high-throughput sequencing platform for 16S rRNA biodiversity sequencing analysis of a total of 10 ileum samples from NT and HS. The experiment includes the total DNA extraction of ileum microbial group, the target fragment was amplified by PCA, and then the amplification product was recovered and purified, the purified product was quantified by fluorescence, the sequencing library was prepared, and the high-throughput sequencing was carried on the MiSeq sequencing instrument.

According to the results of Illumina MiSeq sequencing, the two-terminal sequences through quality screening were paired connection according to overlapping bases by FLASH software. Then the connected sequences were identified and assigned to the corresponding samples according to the corresponding index information of each sample, so as to obtain the effective sequences of each sample. QIIME software was used to eliminate the doubt sequences and count the number of sequences of the above effective sequences, so as to obtain the sequences that can be used for subsequent analysis. Using QIIME software, a sequence alignment tool (Edgar, 2010), the sequences obtained above were merged and divided into OTUs according to 97% sequence similarity, and the sequences with the highest abundance in each OTUs were selected as the representative sequences of the OTUs. The representative sequences of OTUs were identified and classified. According to the obtained OTU abundance matrix, R software was used to calculate the number of common OTUs in NT and HS groups, and the proportion of common and unique OTUs in each group was visually presented through Venn diagram. Chao1, Shannon, Simpson and ACE indices were calculated by QIIME software (Version 1.7.0). The values of indices can reflect the complexity of the sample community. Alpha diversity of OTU was calculated, and the species diversity curve was displayed to obtain the information of species richness and evenness in the sample. Principal component analysis (PCA) was performed on the community composition structure at the genus level by using R software, and the natural distribution characteristics between samples were described by two-dimensional images. Through Metastats, the richness of taxa at the level of phylum, class, order, family, genus and species between samples or groups was statistically compared and displayed the form of violin plot combined with box plot (White et al., 2009). The GraPhlAn visualisation tool was used to construct a hierarchical tree for the composition of the sample at each classification level (Asnicar et al., 2015). At the same time, each classification unit was distinguished by different colours, and their abundance distribution was reflected by the node size, so as to quickly find the dominant microbial groups. The community composition data at each classification level were clustered according to the abundance distribution of classification units or the similarity between samples, and the classification units and samples were sorted according to the clustering results. The R software was used to cluster the top 50 genera of abundance and draw the heat map. Linear discriminant analysis effect size (LEfSe) was performed using default parameters to detect groups with rich differences between groups (Segata et al., 2011). Partial least squares discriminant analysis (PLS-DA) was also introduced as a supervised model, using R software to reveal the microbial flora differences between groups. Using PICRUSt, 16S rRNA gene sequences can be predicted in KEGG functional spectrum database. According to the prediction results of PICRUSt, the annotation information of each sample corresponding to each functional spectrum database can be obtained, and the abundance matrix of the predicted functional groups can be obtained. Then

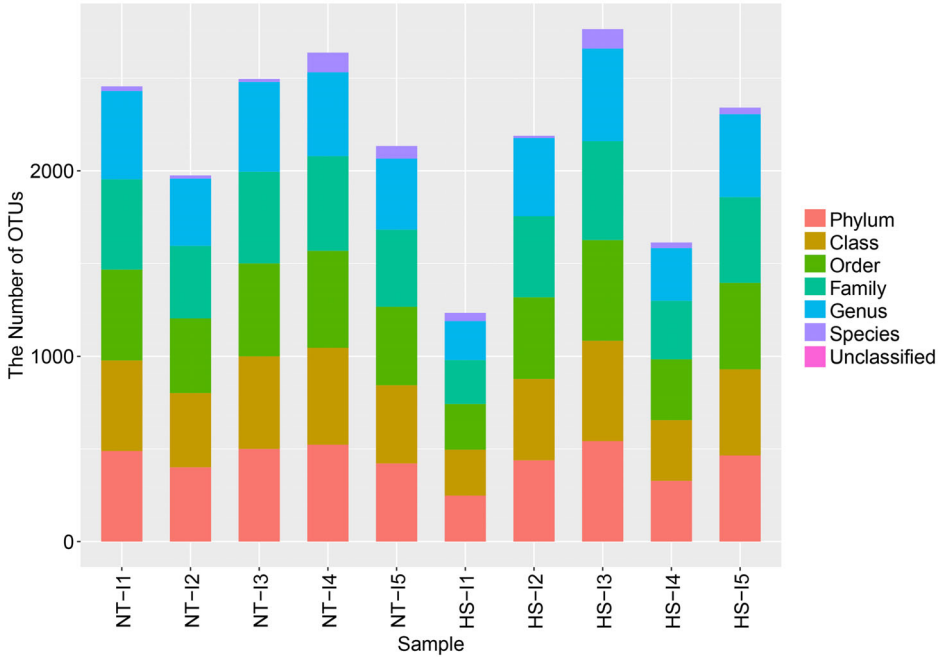

FIGURE 1 OTU classification and classification status identification results statistical chart. Abscissa is arranged according to the sample name, and the ordinate is the number of OTUs that can be classified into phylum, class, order, family, genus and species in each sample. NT, normal temperature group (21.3 ± 1.2°C); HS, heat stress group (32.5 ± 1.4°C)

the abundance of the predicted functional groups in each sample can be displayed in the form of violin plot combined with box plot.

2.4 | Statistical analysis

The experiment uses QIIME software to divide OUT and draw sparse curves. Statistical algorithm of Metastats in Mothur software was used to determine the difference in sequence amount (relative abundance) of each taxonomic at the phylum and genus level between groups. The relative abundance matrix of the genus level submitted to the online analysis platform was LEfSe scored by Galaxy. R software was used to perform PCA analysis, draw heat map, and build PLS-DA discriminant model. PICRUSt was used to predict the function of the tested gene sequence. Alpha diversity data and the data of statistics of the number of microbial groups at each classification level were expressed as $X \pm S$ and analysed by SPSS 16.0. One-way analysis of variance and *t*-test were used to compare the differences of alpha diversity and key flora between groups. $p < 0.05$ indicated that the difference was statistically significant.

3 | RESULTS

3.1 | Operational taxonomic unit partition and classification

Figure 1 presented the results of OTUs partition and classification status identification. There were no significant differences in the number

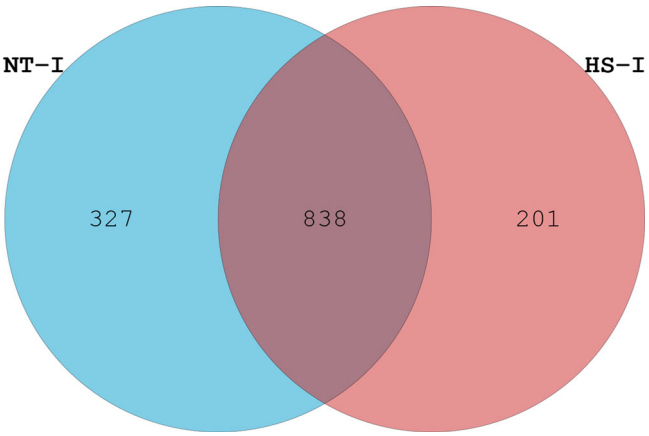

FIGURE 2 Venn diagram which shows the proportion of common and unique OTUs in each group. Blue represents HS group and orange represents NT group. NT, normal temperature group (21.3 ± 1.2°C); HS, heat stress group (32.5 ± 1.4°C)

of OTUs at six different classification levels between the NT group and the HS group ($p > 0.05$). There were 838 common OTUs between the NT group and the and HS group. The NT group acquired 327 OTUs, whereas 201 OTUs from the HS group were acquired (Figure 2).

3.2 | Alpha diversity analysis

The alpha diversity index, including Simpson, Chao1, ACE and Shannon index, was presented in Table 2, and the results showed that HS

TABLE 2 Effect of heat stress on the diversity index of ileal microflora of indigenous yellow-feather broilers

	NT	HS	*p* Value
Simpson	0.85 ± 0.04	0.71 ± 0.11	0.251
Chao 1	507.78 ± 25.60	447.57 ± 58.78	0.375
ACE	528.02 ± 28.56	466.09 ± 60.69	0.382
Shannon	4.74 ± 0.29	3.83 ± 0.67	0.246

NT, normal temperature group (21.3 ± 1.2°C); HS, heat stress group (32.5 ± 1.4°C). $p < 0.05$ was considered to be statistically significant.

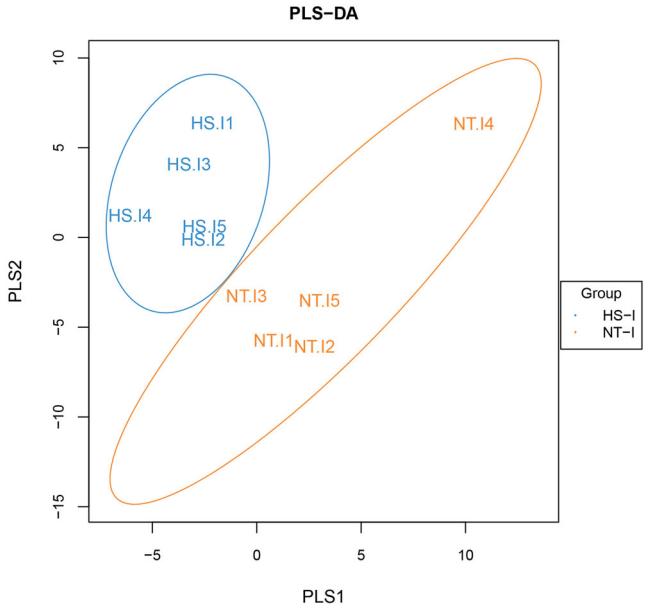

FIGURE 3 PLS-DA discriminant analysis diagram. Each point represents a sample. Blue represents HS group and orange represents NT group. Points with the same colour were marked with ellipses. If the samples belonging to the same group were closer to each other, and the distance between points in different groups is farther, the classification model is good. NT, normal temperature group (21.3 ± 1.2°C); HS, heat stress group (32.5 ± 1.4°C)

exposure had no significant effect on these alpha diversity index of ileal microflora in broilers ($p > 0.05$).

3.3 | Beta diversity analysis

The results of the beta diversity analysis were shown in Figure 3. The distance between samples in NT group was close, so was HS group. But the distance between NT group and HS group was far, which means that the classification model was better.

3.4 | Analysis of taxonomic composition

As described in Table 3, compared with NT, HS had no significant effect on the abundance of ileal microflora at the six levels including phylum, class, order, family, genus and species ($p > 0.05$). Figure 4a shows that at the phylum level, compared with the NT, HS increased the relative abundance of *Firmiaicutes* and *Thermi* in the ileum and decreased the relative abundance of *Proteobacteria*, *Actinobacteria* and *Bacteriodetes*, but both of them did not reach a significant level ($p > 0.05$). At the class level, after HS treatment, the abundances of *Bacilli* and *Alphaproteobacter* increased, while the relative abundances of *Gammaproteobactia*, *Clostridia* and *Alphaoroteobacteria* decreased (Figure 4b), but both of them did not reach a significant level ($p > 0.05$). As shown in Figure 4c, compared with the NT group at the order level, HS increased the abundance of *Lactobacillales*, *Campylobacterales* and *Actinomycetalesd*, and decreased the abundance of *Clostridiales*, *Ehterobacteriales*, *Sphingomonadales*, *Pseadomnadales* and *Pasteurellales*, but both of them not reached a significant level ($p > 0.05$). At the family level (Figure 4d), compared with NT, HS increased the relative abundance of *Lactobacillales* and *Helicobacteraceae*, and decreased the relative abundance of *Clostridiales*, *Enterobacteriaceae*, *Oxalobacteracease*, *Sphingomonadaceae*, *Pasteurellacease*, *Moraxellaceae* and *Enterococcaceae*, but both of them did not reach a significant level ($p > 0.05$). Finally at the genus level (Figure 4e), compared with NT, HS increased the abundance of *Lactobacillus*, and decreased the relative abundance of *Candidatus-Arthromitus*, *Gallibacterium*, *Eenterococcus* and *Campylobacter*, but both of them did not reach a significant level ($p > 0.05$).

TABLE 3 Effect of heat stress on the number of microbial groups in different levels of ileal microflora of indigenous yellow-feather broilers

	NT	HS	*p* Value
Phylum	6.80 ± 0.58	7.2 ± 0.73	0.681
Class	13.00 ± 1.73	13.20 ± 1.50	0.993
Order	20.80 ± 2.03	20.80 ± 2.03	1.000
Family	31.00 ± 5.26	32.60 ± 4.05	0.816
Genus	32.80 ± 6.26	30.20 ± 4.75	0.749
Species	14.60 ± 2.93	12.00 ± 1.79	0.470

NT, normal temperature group (21.3 ± 1.2°C); HS, heat stress group (32.5 ± 1.4°C). $p < 0.05$ was considered to be statistically significant.

Using the statistical algorithm of Metastats, the difference analysis of the sequence number (i.e. absolute abundance) between NT and HS at the genus and genus level for each taxa was shown in Figure 5. At the genus level, the abundance of *Campylobacter* in NT was significantly higher than that in HS ($p < 0.05$).

As shown in Figure 6, at the genus level were significant differences between NT and HS in *Campylobacter*, *Campylobacter*, *Comamonadaceae* and *Delfia*. The bacterial abundance of *Campylobacter* and *Campylobacter* in NT was significantly higher than that in HS ($p < 0.05$). The abundance of *Comamonadaceae* and *Delftia* in NT was significantly lower than that in HS ($p < 0.05$).

Using GraPhlAn software to build a hierarchical tree, as shown in Figure 7 indicating that *Fimicutes*, *Bacilli*, *Lactobacillales*, and *Lactobacillus* were the advantage groups, and following groups were

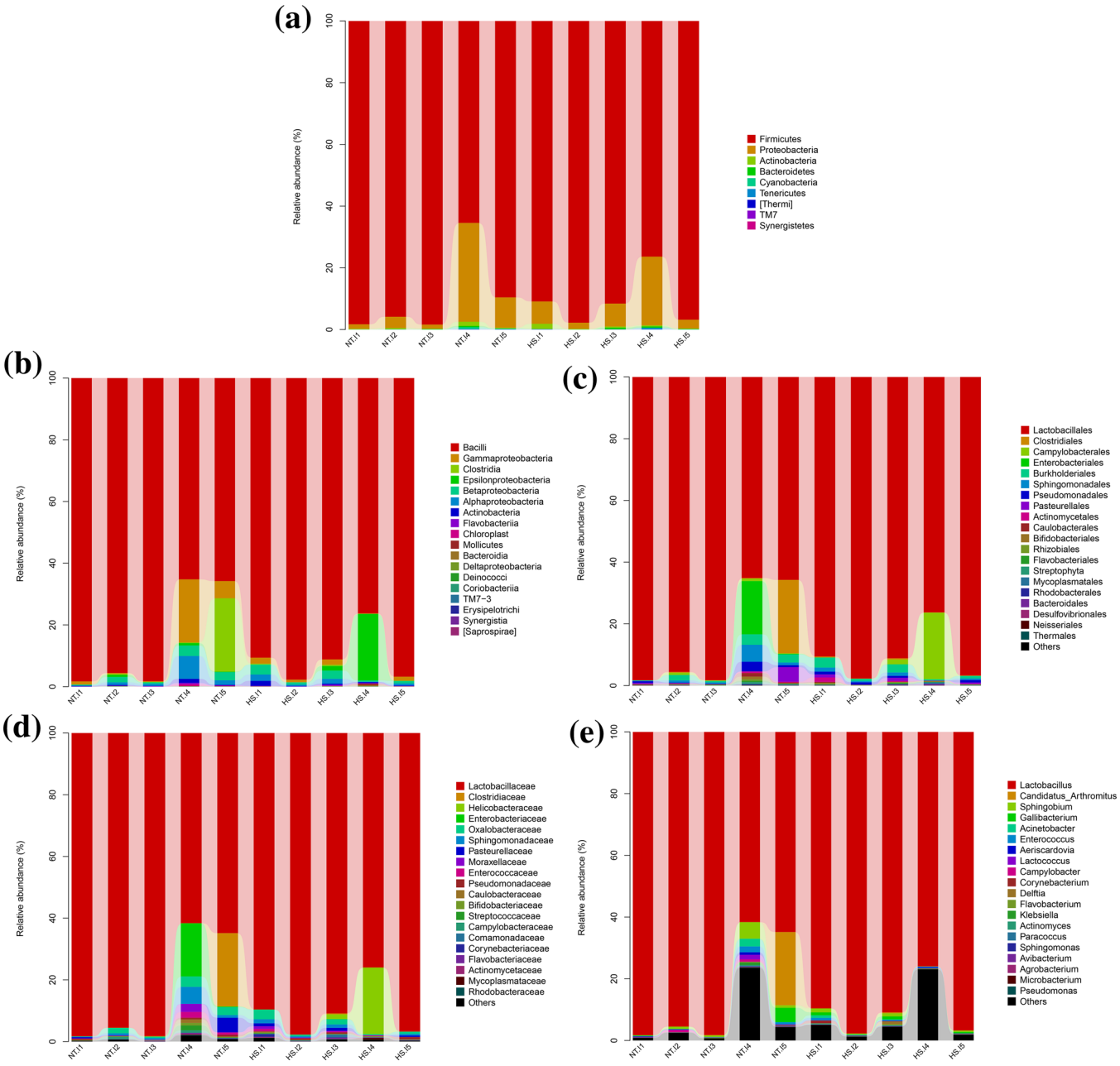

FIGURE 4 (a)–(e) Taxonomic composition and distribution map of ileal microflora. The abscissa is arranged according to the name of the sample, each column represents a sample, and each taxonomic unit is distinguished by colour. The ordinate represents the relative abundance of each taxonomic unit. NT, normal temperature group (21.3 ± 1.2°C); HS, heat stress group (32.5 ± 1.4°C)

Proteobaacteria. These bacteria could be used as the key strains to further explore the effect of HS on intestinal microorganisms.

As shown in Figure 8, compared with NT group, HS reduced the abundance of *Lactococcus*, *Erwinia*, *Herbiconiux* and *Aurantimonas*, and increased the abundance of *Bacteroides*, *Desufovibrio*, *Delftia*, *Bdellovibrio*, *Corynebacterium*, *Rothia*, *Bombwasardovia* and *Astioccacualwas*, but both of them did not reach a significant level ($p > 0.05$).

3.5 | Prediction of metabolic function of microflora

As shown in Figure 9, compared with NT, HS enhanced the abundance of xenobiotics biodegradation and metabolism, lipid metabolism and carbohydrate metabolism in the ileum of Huaixiang chickens. HS could also weaken the nucleotide metabolism and amino acid metabolism of the ileal microflora.

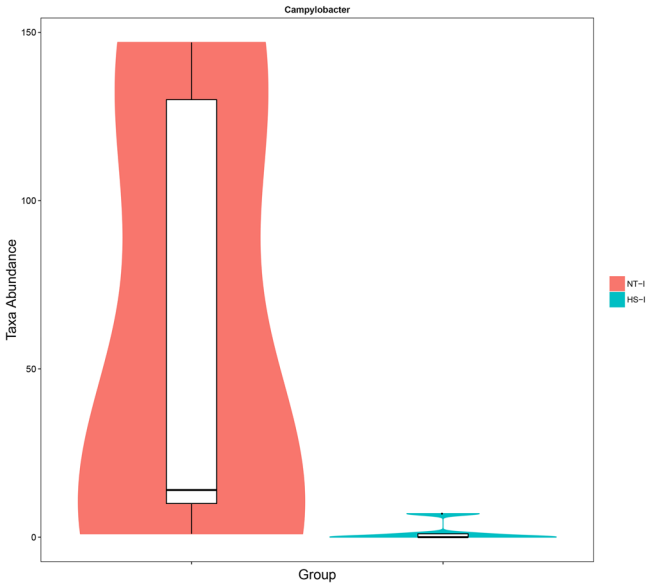

FIGURE 5 The abundance distribution map of the taxa with the most significant differences in Metastats between groups. The violin chart can visually display the distribution characteristics of the data. The 'fat and thin' of the 'violin' reflects the density of the sample data distribution (the wider the width, the more samples corresponding to the sequence amount). Red represents the NT group, and blue represents the HS group. NT, normal temperature group (21.3 ± 1.2°C); HS, heat stress group (32.5 ± 1.4°C)

4 | DISCUSSION

As we know, HS has a series of adverse effects on the intestinal function of broilers, including destroying the physical barrier of the intestine, disturbing the balance of microflora and affecting the gut digestion and absorption (Nosrati et al., 2017; Liu et al., 2021a). In recent years, with the development of intestinal microbial identification technologies such as 16S rRNA, researchers' attention to intestinal microbes has increased (He et al., 2013). This study is based on 16S rRNA sequencing technology, using high-temperature environment as a harmful factor to explore the impact of HS on local yellow-feather broiler breed (Huaixiang chicken). This research showed that the specific OTUs number of the HS group is relatively low compared with the NT group, indicating that HS exposure reduced the diversity of the ileal microflora. Peng (2016) demonstrated that 31°C HS exposure could reduce the diversity of the ileal microflora in broilers. However, in the research report of Li et al. (2016), HS has greater influence on the ileal microflora of Rizhao chickens than other intestinal microflora, and the abundance and diversity of the microflora in the ileum will increase with the increase of HS time. Therefore, the results of previous studies were not consistent. This may be related to the broiler breeds, growth stages and feed formula (Wang et al., 2020).

In this experiment, although HS exposure had no significant effect on alpha diversity of the ileal microflora; however, the broilers in HS group had lower Shannon, Simpson, Chao1 and ACE index than those in NT group, which is consistent with the OTU results. Similarly, Xiong et al. (2020) suggested that the Simpson index of pig manure in the HS group was significantly lower than that of the NT group, while the Shannon index was not different. Sohail et al. (2015) also reported that HS exposure had profound effects on the species richness of cecal microflora in broilers, but it did not obviously influence the alpha diversity. At the same times, Xing et al. (2019) found that cyclic HS had no significant effect on the species richness and alpha diversity of the cecal microflora in laying hens. But, Hsieh et al. (2017) studied the effect of HS on the cecal microflora of laying hens at different growth stages, and the alpha diversity of cecal microflora began to increase after 2 weeks of HS exposure and then reached a significant level after 3 weeks of HS exposure. Wang et al. (2018) reported that the species richness of broilers' ileal microflora increased significantly after 14 days of HS. Therefore, the effects of HS on gut microflora of broilers are complex and changeable, and the experimental results were not completely consistent. Actually, previous study also has indicated that the changes of the intestinal microflora were not exactly the same due to the influence of extent and duration of HS (Khosravl, 2013). Moreover, some studies have demonstrated that the effect of HS on the alpha diversity of broiler ileum microflora is mainly related to the reduction of feed intake (Wang, 2019). Therefore, the diet types, growth stages, stress intensity and duration, broiler strains and the different intestinal segments could explain the variable effects of HS on the intestinal microbial structure in broilers.

Regarding the taxonomic composition of the ileal microflora, the present study found that the abundance of *Campylobacter* was significantly reduced by HS, while the abundance of *Delftia* was significantly increased by HS. Previous studies have shown that *Delftia*, as a non-pathogenic environmental organism, is commonly found in potentially malignant tumours patients with low immune function (Hagiya et al., 2013; Mei et al., 2018; Ranc et al., 2018). This shows that *Delftia* is pathogenic to the body with low immunity. HS causes decreased feed intake, enhanced metabolism and decreased immune function of livestock and poultry, which ultimately results in decreased growth, production and reproductive performance of livestock and poultry (Goo et al., 2019; Mohamed et al., 2019; Sejian et al., 2018). This gave *Delftia* an opportunity; that is, the amount of *Delftia* in the ileum of Huaixiang chickens increased significantly after HS. The changes in the number of *Campylobacters* can be linked to the prediction results of the intestinal microflora metabolic function in this test. The test results show that HS will reduce the amino acid metabolism function of the microflora, while the high carbohydrate metabolism function. Surprisingly, *Campylobacter* lacks the ability to use many common carbohydrates as carbon sources but can effectively use citric acid cycle intermediates and various amino acids, especially closely related with aspartic acid, glutamic acid, serine and proline acid metabolism (Parkhill et al., 2000; Stahl et al., 2012). Therefore, the decrease in the abundance of *Campylobacter* in the ileum of Huaixiang chickens under high temperature is likely to be related to the decrease in the metabolic function of the microflora caused by HS. In addition, we have also noticed that the lipid metabolism function of the flora in the ileum of Huaixiang chickens will be significantly reduced under the influence of high-temperature environment. Previous studies have shown that HS

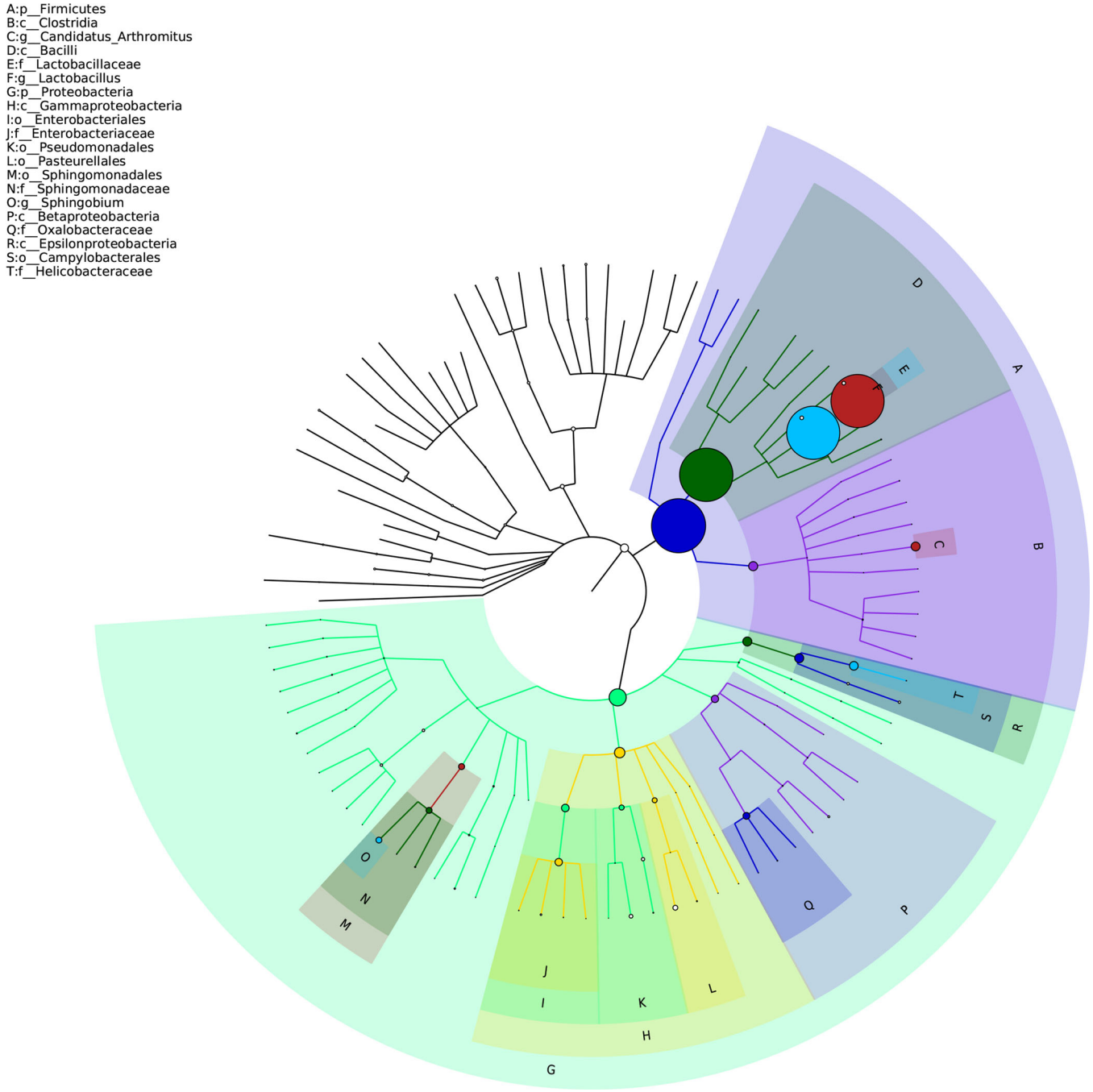

FIGURE 6 Intergroup difference classification unit display based on classification hierarchy tree. The longer the length, the more significant the difference of the taxon; the red represents the NT group, and the green represents the HS group. NT, normal temperature group (21.3 ± 1.2°C); HS, heat stress group (32.5 ± 1.4°C)

can reduce feed intake and increase energy consumption in broilers. In order to alleviate this phenomenon, the body will enhance the mobilisation of glycogen and amino acids, regulate key gluconeogenic enzymes to promote their metabolism and promote fat synthesis and deposition, thereby alleviating the negative balance of energy metabolism (Akşlt et al., 2006; Guo et al., 2020; Habashy et al., 2017; Ma et al., 2021). Lu et al. (2018) believed that the way to alleviate the negative energy balance will lead to changes in fatty acid biosynthesis pathways and cause lipid metabolism disorders. Combined with this experiment, changes in the lipid metabolism function of the intestinal microflora can also be one of the directions for future exploration of HS-induced lipid metabolism disorders. This series of changes reminds that the high-temperature environment has an interlocking influence on the various systems of broilers. When exploring the effects of HS on

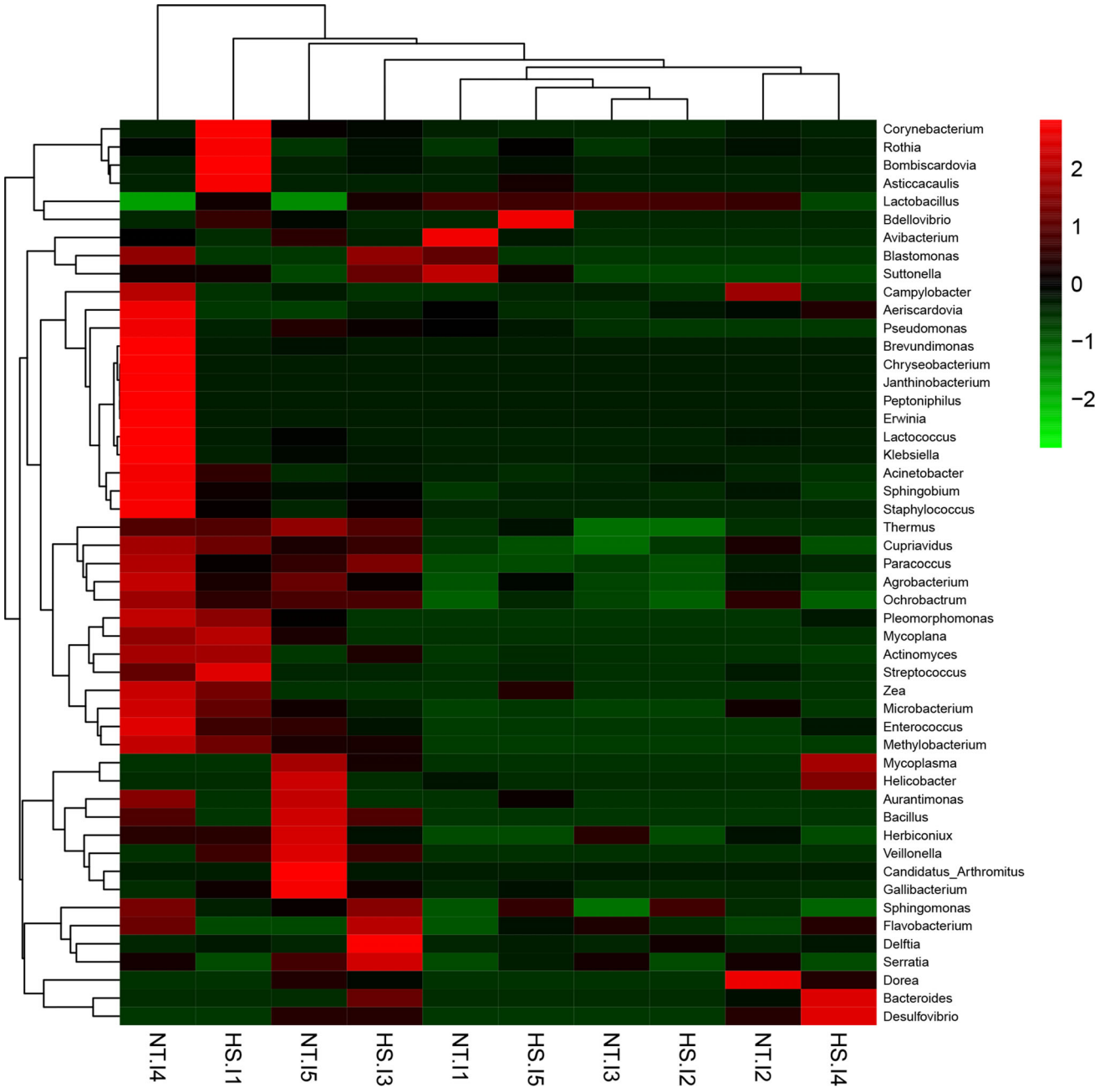

FIGURE 7 GraPhlAn sample overall classification tree. The classification hierarchy tree shows the top 20 taxa with relative abundance in the sample, and the hierarchical relationship of all taxa (indicated by nodes) from phylum to genera (arranged from inner circle to OTUs circle), and the size of the node corresponds to the taxa average relative abundance. NT, normal temperature group (21.3 ± 1.2°C); HS, heat stress group (32.5 ± 1.4°C)

broilers, we should consider the other side from multiple angles and cannot be single.

5 | CONCLUSIONS

In conclusion, this study showed that yellow-feather broilers (Huaixiang chickens) were exposed to heat stress for 4 weeks, the abundance of *Campylobacter* in the ileum decreased and the abundance of *Delftia* in the ileum increased. The alpha diversity of ileal microbiota was not significantly affected by HS exposure. These findings were beneficial to further researches on the effects of HS on intestinal health in slow-growing yellow-feather broilers.

ETHICAL STATEMENT

The Guangdong Ocean University's Animal Ethics Committee (Zhanjiang, China) approved the protocol of broilers feeding, care and sampling in this study.

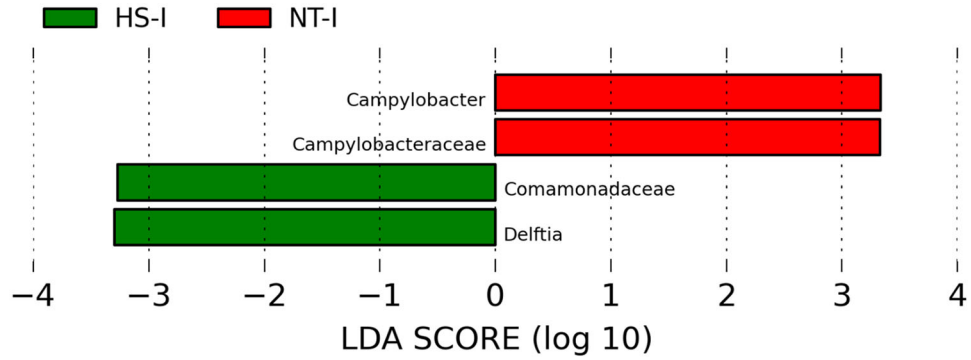

FIGURE 8 Heat map of community composition at genus level combined with cluster analysis. Red represents the genera with higher abundance in the corresponding sample, and green represents the genera with lower abundance. NT, normal temperature group (21.3 ± 1.2°C); HS, heat stress group (32.5 ± 1.4°C)

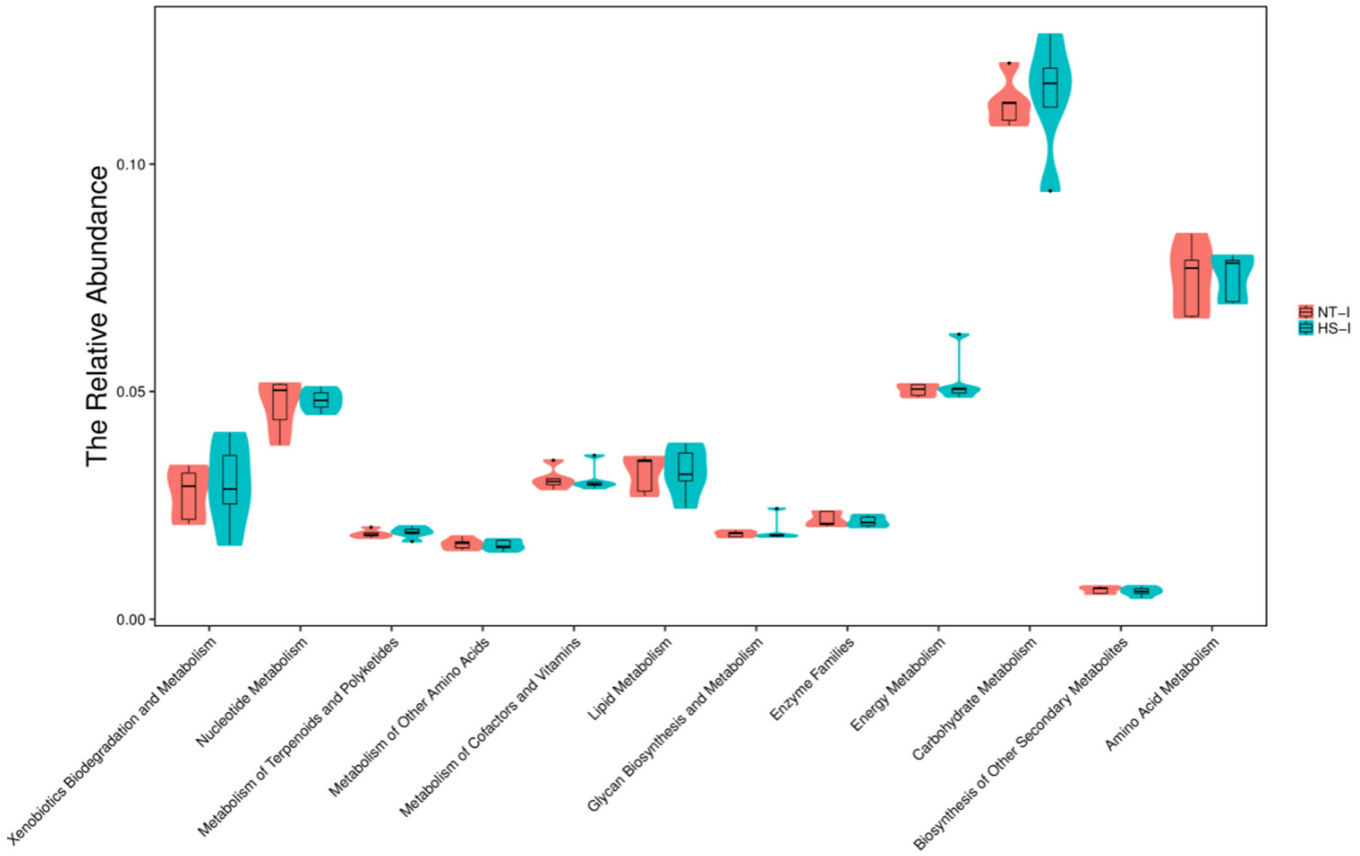

FIGURE 9 PICRUSt predicted KEGG second-level distribution map. The 'fat and thin' of 'violin' reflects the density of the sample data distribution (the wider the width, the more samples corresponding to the abundance). NT, normal temperature group (21.3 ± 1.2°C); HS, heat stress group (32.5 ± 1.4°C)

AUTHOR CONTRIBUTIONS

Yong-Yan Jin: data curation; formal analysis; writing – original draft; writing – review & editing. **Yan Guo**: formal analysis; methodology; writing – review & editing. **Chun-Tian Zheng**: conceptualisation; project administration; supervision; writing – review & editing. **Wen-Chao Liu**: conceptualisation; funding acquisition; methodology; project administration; supervision; writing – review & editing.

CONFLICT OF INTEREST

The authors report that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study were available from the corresponding author upon reasonable request.

REFERENCES

Akşit, M., Yalçin, S., Ozkan, S., Metin, K., & Ozdemir, D. (2006). Effects of temperature during rearing and crating on stress parameters and meat quality of broilers. *Poultry Science*, *85*(11), 1867–1874.

Asnicar, F., Weingart, G., Tickle, T. L., Huttenhower, C., & Segata, N. (2015). Compact graphical representation of phylogenetic data and metadata with GraPhlAn. *Peer J*, *3*, e1029.

Chauhan, S. S., Rashamol, V. P., Bagath, M., Sejian, V., & Dunshea, F. R. (2021). Impacts of HS on immune responses and oxidative stress in farm animals and nutritional strategies for amelioration. *International Journal of Biometeorology*, *65*(7), 1231–1244.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than Blast. *Bioinformatics*, *26*(19), 2460–2461.

Emami, N. K., Jung, U., Voy, B., & Dridi, S. (2021). Radical response: Effects of HS-induced oxidative stress on lipid metabolism in the avian liver. *Antioxidants*, *10*(1), 35.

Goo, D., Kim, H. J., Park, G. H., Delos Reyes, J B., & Kill, D. Y. (2019). Effect of HS and stocking density on growth performance, breast meat quality, and intestinal barrier function in broiler chickens. *Animals*, *9*(3), 107.

Guo, Y., Zhao, Z. H., Pan, Z. Y., An, L. L., & Liu, W. C. (2020). New insights into the role of dietary marine-derived polysaccharides on productive performance, egg quality, antioxidant capacity, and jejunal morphology in late-phase laying hens. *Poultry Science*, *99*(4), 2100–2107.

Guo, Y., Balasubramanian, B., Zhao, Z. H., & Liu, W. C. (2021a). Heat stress alters serum lipid metabolism of Chinese indigenous broiler chickens—A lipidomics study. *Environmental Science and Pollution Research International*, *28*(9), 10707–10717.

Guo, Y., Balasubramanian, B., Zhao, Z. H., & Liu, W. C. (2021b). Marine algal polysaccharides alleviate aflatoxin B1-induced bursa of Fabricius injury by regulating redox and apoptotic signaling pathway in broilers. *Poultry Science*, *100*(2), 844–857.

Guo, Y., Liao, J. H., Liang, Z. L., Balasubramanian, B., & Liu, W. C. (2021c). Hepatic lipid metabolomics in response to heat stress in local broiler chickens breed (Huaixiang chickens). *Veterinary Medicine and Science*, *7*, 1369–1378.

Habashy, W. S., Milfort, M. C., Fuller, A. L., Attia, Y. A., Rekaya, R., & Aggrey, S. E. (2017). Effect of HS on protein utilization and nutrient transporters in meat-type chickens. *International Journal of Biometeorology*, *61*(12), 2111–2118.

Hagiya, H., Murase, T., Sugiyama, J., Kuroe, Y., Nojima, H., Naito, H., Hagioka, S., & Morimoto, N. (2013). Delftia acidovorans bacteremia caused by bacterial translocation after organophosphorus poisoning in an immunocompetent adult patient. *Journal of Infection and Chemotherapy*, *19*(2), 338–341.

He, Y., Zhou, B. J., Deng, G. H., Jiang, X. T., Zhang, H., & Zhou, H. W. (2013). Comparison of microbial diversity determined with the same variable tag sequence extracted from two different PCR amplicons. *BMC Microbiology*, *13*, 208.

Hirakawa, R., Nurjanah, S., Furukawa, K., Murai, A., Kikusato, M., Nochi, T., & Toyomizu, M. (2020). HS causes immune abnormalities via massive damage to effect proliferation and differentiation of lymphocytes in broiler chickens. *Frontiers in Veterinary Science*, *7*, 46.

Hsieh, A. N., Looft, T., Persia, M. E., & Lamont, S. J. (2017). Cecal microbiome characterization for layers under HS. *Animal Industry Report*, *663*, 52.

Hudak, J. E., Alvwerez, D., Skelly, A., Von Andrian, U. H., & Kasper, D. L. (2017). Illuminating vital surface molecules of symbionts in health and disease. *Nature Microbiology*, *2*, 17099.

Kadykalo, S., Roberts, T., Thompson, M., Wilson, J., Lang, M., & Espeisse, O. (2018). The value of anticoccidials for sustainable global poultry production. *International Journal of Antimicrobial Agents*, *51*(3), 304–310.

Kaldhusdal, M., Benestad, S. L., & Løvland, A. (2016). Epidemiologic aspects of necrotic enteritis in broiler chickens-disease occurrence and production performance. *Avian Pathology*, *45*(3), 271–274.

Khosravi, A., & Mazmanian, S. K. (2013). Disruption of the gut microbiome as a risk factor for microbial infections. *Current Opinion in Microbiology*, *16*(2), 221–227.

Li, Y. Z., Chen, C. X., Jin, Z. L., & Zheng, Z. H. (2016). Correlation analysis of intestinal microflora diversity and mucosal structure in chickens under HS. *Journal of China Agricultural University*, *1*, 71–80.

Liang, Z. L., Jin, Y. Y., Guo, Y., Qiu, S. J., Zhao, Y., Zhao, Z. H., & Liu, W. C. (2021). Heat stress affects duodenal microbial community of indigenous yellow-feather broilers as determined by 16S rRNA sequencing. *Italian Journal of Animal Science*, *20*, 1222–1231.

Liu, W. C., Guo, Y., Zhao, Z. H., Jha R., & Balasubramanian B. (2020). Algae-derived polysaccharides promote growth performance by improving antioxidant capacity and intestinal barrier function in broiler chickens. *Frontiers in Veterinary Science*, *7*, 601336.

Liu, W., Yuan, Y., Sun, C., Balamuralikrishnan, B., Zhao, Z., & An, L. (2019). Effects of dietary betaine on growth performance, digestive function, carcass traits, and meat quality in indigenous yellow-feathered broilers under long-term heat stress. *Animals*, *9*(8), 506.

Liu, W. C., Guo, Y., An, L. L., & Zhao, Z. H. (2021a). Protective effects of dietary betaine on intestinal barrier function and cecal microbial community in indigenous broiler chickens exposed to high temperature environment. *Environmental Science and Pollution Research*, *28*(9), 10860–10871.

Liu, W. C., Ou, B. H., Liang, Z. L., Zhang, R., & Zhao, Z. H. (2021b). Algae-derived polysaccharides supplementation ameliorates HS-induced impairment of bursa of Fabricius via modulating NF-κB signaling pathway in broilers. *Poultry Science*, *100*(8), 101139.

Liu, W. C., Zhu, Y. R., Zhao, Z. H., Jiang, P., & Yin, F. Q. (2021c). Effects of dietary supplementation of algae-derived polysaccharides on morphology, tight junctions, antioxidant capacity and immune response of duodenum in broilers under heat stress. *Animals*, *11*(8), 2279.

Lu, Z., He, X., Ma, B., Zhang, L., Li, J., Jiang, Y., Zhou, G., & Gao, F. (2018). Serum metabolomics study of nutrient metabolic variations in chronic heat-stressed broilers. *British Journal of Nutrition*, *119*(7), 771–781.

Ma, B., Zhang, L., Li, J., Xing, T., Jiang, Y., & Gao, F. (2021). HS alters muscle protein and amino acid metabolism and accelerates liver gluconeogenesis for energy supply in broilers. *Poultry Science*, *100*(1), 215–223.

Mei, Q. X., Huang, C. L., Luo, S. Z., Zhang, X. M., Zeng, Y., & Lu, Y. Y. (2018). Characterization of the duodenal bacterial microbiota in patients with pancreatic head cancer vs. healthy controls. *Pancreatology*, *18*(4), 438–445.

Meng, X., Zhang, Y., Botchway, B. O. A., Zhou, J., Xu, L., & Liu, X. (2018). Role of AMP activated protein kinase signaling pathway in intestinal development of mammals. *Annals of Anatomy*, *220*, 51–54.

Mohamed, A. S. A., Lozovski, A. R., & Ali, A. M. A. (2019). Nutritional strategies to alleviate HS effects through feed restrictions. *Journal of Animal Behaviour and Biometeorology*, *7*(3), 123–131.

Mohamed, M. A. D., & Suhair, A. A. (2018). Attenuating effects of caffeic acid phenethyl ester and betaine on abamectin-induced hepatotoxicity and nephrotoxicity. *Environmental Science and Pollution Research*, *25*(16), 1–9.

Najafi, P., Zulkifli, I., Jajuli, N. A., Farjam, A. S., Ramiah, S. K., Amir, A. A., O'Reily, E., & Eckersall, D. (2015). Environmental temperature and stocking density effects on acute phase proteins, heat shock protein 70, circulating corticosterone and performance in broiler chickens. *International Journal of Biometeorology*, *59*(11), 1577–1583.

Nosrati, M., Javandel, F., Camacho, L. M., Khusro, A., Cipriano, M., Seidavi, A. R., & Salem, A. Z. M. (2017). The effects of antibiotic, probiotic, organic acid, vitamin C, and Echinacea purpurea extract on performance, carcass characteristics, blood chemistry, microbiota, and immunity of broiler chickens. *Journal of Applied Poultry Research*, *26*(2), 295–306.

Pan, D., & Yu, Z. (2014). Intestinal microbiome of poultry and its interaction with host and diet. *Gut Microbes*, *5*(1), 108–119.

Parkhill, J., Wren, B. W., Mungall, K., Ketley, J. M., Churcher, C., Basham, D., Chillingworth, T., Davies, R. M., Feltwell, T., Holroyd, S., Jagels, K., Karlyshev, A. V., Moule, S., Pallen, M. J., Penn, C. W., Quail, M. A., Rajandream, M. A., Rutherford, K. M., Vliet, A. H., Whitehead, S., & Barrell, B. G. (2000). The genome sequence of the food-borne pathogen Campylobacter jejuni reveals hypervariable sequences. *Nature*, *403*(6770), 665–668.

Peng, Q. Q. (2016) *Effects of different thermal environments on bacterial diversity and nitrogen metabolism in cecum of broilers*. Hebei University of Engineering, Thesis.

Quinteiro-Filho, W. M., Ribeiro, A., Ferraz-de-Paula, V., Pinheiro, M. L., Sakai, M., Sá, L. R., Ferreira, A. J., & Palermo-Neto, J. (2010). HS impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. *Poultry Science*, *89*(9), 1905–1914.

Ranc, A., Dubourg, G., Fournier, P. E., Raoult, D., & Fenollar, F. (2018). *Delftia tsuruhatensis*, an emergent opportunistic healthcwere-associated pathogen. *Emerging Infectious Diseases Journal*, *24*(3), 594–596.

Richards, M. P., Rosebrough, R. W., Coon, C. N., & McMurtry, J. P. (2010). Feed intake regulation for the female broiler breeder: In theory and in practice. *Journal of Applied Poultry*, *19*(2), 182–193.

Rostagno, M. H. (2020). Effects of HS on the gut health of poultry. *Journal of Animal Science*, *98*(4), skaa090.

Round, J. L., Lee, S. M., Li, J., Tran, G., Jabri, B., Chatila, T. A., & Mazmanian, S. K. (2011). The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science*, *332*(6032), 974–977.

Sejian, V., Bhatta, R., Gaughan, J. B., Dunshea, F. R., & Lacetera, N. (2018). Review: Adaptation of animals to HS. *Animal*, *12*(s2),s431-s444.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, *12*(6), R60.

Serino, M., Luche, E., Gres, S., Baylac, A., Bergé, M., Cenac, C., Waget, A., Klopp, P., Iacovoni, J., Klopp, C., Mariette, J., Bouchez, O., Lluch, J., Ouarné, F., Monsan, P., Valet, P., Roques, C., Amar, J., Bouloumié, A., Théodorou, V., & Burcelin, R. (2012). Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. *Gut*, *61*(4), 543–553.

Shaufi, M. A. M., Sieo, C. C., Chong, C. W., Gan, H. M., & Yin, W. H. (2015). Deciphering chicken gut microbial dynamics based on high-throughput 16S rRNA metagenomics analyses. *Gut Pathogens*, *7*, 4.

She, Y., Cai, H., & Liu, G. (2018). Effects of antibiotic on microflora in ileum and cecum for broilers by 16S rRNA sequence analysis. *Animal Science Journal*, *89*(12), 1680–1691.

Shi, D., Bai, L., Qu, Q., Zhou, S., Yang, M., Guo, S., Li, Q., & Liu, C. (2019). Impact of gut microbiota structure in heat-stressed broilers. *Poultry Science*, *98*(6), 2405–2413.

Skinner, J. T., Bauer, S., Young, V., Pauling, G., & Wilson, J. (2010). An economic analysis of the impact of subclinical (mild) necrotic enteritis in broiler chickens. *Avian Diseases*, *54*(4), 1237–1240.

Sohail, M. U., Hume, M. E., Byrd, J. A., Nisbet, D. J., Shabbir, M. Z., Ijaz, A., & Rehman, H. (2015). Molecular analysis of the ileal and tracheal microbiome of heat-stressed broilers supplemented with prebiotic and probiotic. *Avian Pathology*, *44*(2), 67–74.

Swartz, T. D., Sakar, Y., Duca, F. A., & Covasa, M. (2013). Preserved adiposity in the Fischer 344 rat devoid of gut microbiota. *The Faseb Journal*, *27*(4), 1701–1710.

Stahl, M., Butcher, J., & Stintzi, A. (2012). Nutrient acquisition and metabolism by *Campylobacter jejuni*. *Frontiers in Cellular and Infection Microbiology*, *7*, 2–5.

Thomas, M., Wongkuna, S., Ghimire S., Kumar, R., Antony, L., Doerner, K. C., Singery, A., Nelson, E., Woyengo, T., Chankhamhaengdecha, S., Janvilisri, T., & Scaria, J. (2019). Gut microbial dynamics during conventionalization of germfree chicken. *mSphere*, *4*(2), e00035–19.

Wang, X. J. (2019). Effects of environmental high temperature on ileal mucosal barrier and microbial flora in broilers. *Chinese Academy of Agricultural Sciences*, Thesis.

Wang, X. J., Feng, J. H., Zhang, M. H., Li, X. M., Ma, D. D., & Chang, S. S. (2018). Effects of high ambient temperature on the community structure and composition of ileal microbiome of broilers. *Poultry Science*, *97*(6), 2153–2158.

Wang, Y. D. (2020). Effects of HS on intestinal microflora in chickens and its control measures. *Animal Husbandry and Veterinary Science*, *3*, 44–45.

White, J. R., Nagarajan, N., & Pop, M. (2009). Statistical methods for detecting differentially abundant features in clinical metagenomic samples. *PLOS Computational Biology*, *5*(4), e1000352.

Xing, S., Wang, X., Diao, H., Zhang, M., Zhou, Y., & Feng, J. (2019). Changes in the cecal microbiota of laying hens during HS is mainly associated with reduced feed intake. *Poultry Science*, *98*(11), 5257–5264.

Xiong, Y., Yi, H., Wu, Q., Jiang, Z., & Wang, L. (2020). Effects of acute HS on intestinal microbiota in grow-finishing pigs, and associations with feed intake and serum profile. *Journal of Applied Microbiology*, *128*(3), 840–852.

Yoshimura, K., Morotomi, N., Fukuda, K., Nakano, M., Kashimura, M., Hachisuga, T., & Taniguchi, H. (2011). Intravaginal microbial flora by the 16S rRNA gene sequencing. *American Journal of Obstetrics and Gynecology*, *205*(3), 235e1–9.

Zhao, Y., Balasubramanian, B., Guo, Y., Qiu, S. J., Jha, R., & Liu, W. C. (2021). Dietary Enteromorpha polysaccharides supplementation improves breast muscle yield and is associated with modification of mRNA transcriptome in broiler chickens. *Frontiers in Veterinary Science*, *8*, 663988.

Zhao, R., & Chen, M. W. (2015). Progress in research methods of intestinal microbiome. *Journal of Biological EngineeringK*, *32*, 1150–1154.

Zhu, H. B. (2020). *Effect of chronic heat stress on intestinal mucosal barrier function in broilers*. Hebei University of Engineering.

Zhu, L., Liao, R., Wu, N., Zhu, G., & Yang, C. (2019). HS mediates changes in fecal microbiome and functional pathways of laying hens. *Applied Microbiology and Biotechnology*, *103*(1), 461–472.

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