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A controlled heat stress during late gestation affects thermoregulation, productive performance, and metabolite profiles of primiparous sow

Jianwen He[a], Weijiang Zheng[a,b], Mingzhou Lu[c], Xiaojing Yang[d], Yongqiang Xue[a], Wen Yao[a,b,d,*]

[a] College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, PR China
[b] National Experimental Teaching Center for Animal Science, Nanjing Agricultural University, Nanjing 210095, PR China
[c] College of Engineering, Laboratory of Modern Facility Agriculture Technology and Equipment Engineering of Jiangsu Province, Nanjing Agricultural University, Nanjing 210031, PR China
[d] Key Lab of Animal Physiology and Biochemistry, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Nanjing Agricultural University, Nanjing 210095, PR China

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ABSTRACT

Heat stress (HS) alters metabolic parameters and reduces productive performance in lactating sows. However, the impact of HS on metabolomic profiles of sows during late gestation is not fully understood. We present here, a study investigating the productive performance and metabolic responses in sows when exposed to HS during late gestation. Twelve first-parity Landrace × Large White F1 sows were randomly assigned into two environmental treatments including the thermoneutral (TN) (18–22 °C; n = 6) and HS (28–32 °C; n = 6) conditions from 85 d of gestation until farrowing. Rectal temperature (RT), respiration rates (RR), and surface temperature (ST) were measured every 4 h from 0800 h to 2000 h during the 2nd week. Farrowing and litter Data, as well as duration of eating, were monitored to assess sows' productive performance. Blood biochemical parameters and urinary metabolomic profiles were measured on d107 of gestation to analyze the host metabolic responses. Our results show that HS increased RT, RR, and ST ($P < 0.0001$). Duration of parturition was prolonged during the delivery in HS group ($P < 0.05$). Piglet body weight (BW) at d 10 and weaning were reduced by 18% and 17% respectively due to maternal HS ($P < 0.001$). Duration of eating increased as a result of HS ($P < 0.001$), consistent with the significant changes observed in serum ghrelin ($P < 0.05$). Moreover, serum ACTH, cortisol, insulin, creatinine, and BUN saw increase as well ($P < 0.05$). Plasma NEFA were elevated by HS ($P < 0.001$). Additionally, HS elevated ($VIP > 1$, log_2 *fold change* > 0.585, and $P < 0.05$) the relative concentrations of 5-aminovaleric acid, β-alanine, cysteine, isoleucine, glyceric acid, erythronic acid, mannitol, erythritol, 2-methyl-1,3-butanediol, and pantothenic acid in urine. These ten metabolites mainly affected the pantothenate and CoA biosynthesis, β-alanine metabolism, and glycerolipid metabolism in pregnant sows. In summary, our study suggests that the controlled HS during late gestation elevates thermal responses, reduces productive performance, and more importantly, enhances the catabolism of lipid and protein of first-parity pregnant sow.

1. Introduction

A growing body of evidence indicated that the global climate has been warming (Dunne et al., 2013; Sherwood and Huber, 2010). Specifically, the global temperature rose at an average rate of approximately 0.13 °C per decade over the last 50 years (Min et al., 2017). As a consequence of global warming, heat stress (HS) is predicted to increase its severity in animal production. Among farm animals, sows are particularly sensitive to a hot environment not only because of their lack of effective sweat glands, but also their thick layer of subcutaneous adipose tissue that impedes radiant heat loss (Ross et al., 2015). Moreover, the genetic selection for improved lean tissue accretion rates and reproductive capacity are both accompanied by increased endogenous heat production (Seibert et al., 2018). Recent studies estimated HS-induced poor sow performance costs the USA swine industry an annual $450 million (Pollman, 2010), the economic loss, however, may be further increased if climate gets worse (Hoffmann, 2010).

The previous studies mainly focus on the lactating sows as lactation is a critical period of both high metabolic load, which is more sensitive to environmental temperature, and milk production, which affects offspring growth (Lucy and Safranski, 2017; Williams et al., 2013). HS in the lactation normally reduces sows' feed intake (Renaudeau et al.,

* Corresponding author at: College of Animal Science and Technology, Nanjing Agricultural University, 210095 Nanjing, PR China.
E-mail address: yaowen67jp@njau.edu.cn (W. Yao).

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2012), leading to negative energy balance, greater body condition loss and is associated with various reproductive problems related to inadequate ovarian function (Nardone et al., 2006). Additionally, HS during lactation also reduces milk production, this negatively affects piglet growth and their weaning weight (Quiniou and Noblet, 1999). The impact of HS, however, is not only limited to the lactation period, but also extended to late gestation which is a critical period for animals due to fetal growth and development, and relationship with postpartum milk production (Hawkins et al., 2001; Moore et al., 1992; Omtvedt et al., 1971). The importance of late gestation has been well-documented in dairy cattle, however limited studies were found in pigs. The previous review indicated that the fetus grows at the fastest rate during the late gestation and accumulates approximately 60% of its birth weight in dairy cattle (Tao and Dahl, 2013). More importantly, the transition period from late gestation to early lactation is prone to show immune dysfunction, negative energy balance, and metabolic disorders in dairy cattle (Tao and Dahl, 2013). Given the important physiological impacts on animals during late gestation, an investigation with respect to the effects of HS on sows during late gestation is imperative.

To date, only a few studies focus on the effects of HS on the late gestational sows. Their results suggested that HS increased the number of stillborn piglets (Omtvedt et al., 1971; Wegner et al., 2016) and reduced newborn piglet weight (Lucy et al., 2012). However, information regarding other productive performance and metabolic profiles of sows was not shown. Herein, we present our study investigating the effects of HS during late gestation on productive performance and metabolic profiles of sows. We used primiparous sows that are especially sensitive to HS (Love, 1978), and we hypothesized that HS influences the sows' productive performance by altering metabolic profiles including lipolysis and proteolysis. We started by building the HS model from which the thermoregulatory parameters were determined, followed by data collection including farrowing and litter data, and duration of eating, in order to assess sows' productive performance. Moreover, a combination of blood biochemical parameters and urinary metabolomic profiles to analyze the host metabolic responses were also discussed.

2. Materials and methods

2.1. Ethical approval

All experimental design and procedures of this study were approved by the Animal Care and Use Committee of Nanjing Agricultural University, in compliance with the Regulations for the Administration of Affairs Concerning Experimental Animals of China.

2.2. Animals, facilities, and experimental design

Twelve first-parity Landrace × Large White F1 sows were brought into the Environmental Center in Jingjiang Farm at 82d of gestation. The sows were reared, housed, artificially inseminated and confirmed pregnant before being brought in the Environmental Center. The center contains 2 environmental chambers (each 8 × 8 × 3 m). The chambers were ventilated with 100% outside air that was exhausted to the outside (air was not recycled). Each chamber had 6 farrowing crates (2.1 × 1.8 m; Dahong Agriproducts, Yixing, China) with watering nipples and feeder mounted to the front of the crate. The farrowing crates were erected approximately 0.6 m above the floor and the sows laid on the composite plastic slat. The light cycle in the chambers was 15 h light and 9 h dark. After three days of adjustment, sows were randomly assigned into two environmental treatments containing thermoneutral (TN) (18–22 °C; n = 6) and HS (28–32 °C; n = 6) conditions from 85 d of gestation until farrowing.

2.3. Feeding and feed intake measurements

Sows in late gestation were fed a corn-soybean meal-based diet twice daily at 0700 h and 1630 h (Table 1). Feed offered was recorded by using an electronic scale (model HY-809; Zhizun Equipment Company, Jinhua, China). Since pregnant sows were limit-fed during late gestation, sows were offered 3.0 kg per day and no feed was left. The Duration of eating, the time from offering feeds to finishing eating all the feeds, was used as a measure for feeding behavior. Sows reduced their feed intake owing to the coming parturition during the last three-days before farrowing, which cannot be used to calculate the duration of eating. Additionally, in order to better understand the eating behavior of pregnant sows under HS condition, eating efficiency was calculated as the ratio of the true eating time to the whole eating duration. This data was based on the video recorded using a digital camera (model C3W; Hikvision Digital Technology Co., Ltd, Zhejiang, China) in the six days (d8, d10, d12, d14, d16, d18) of the trial.

Table 1
Composition and nutrient levels of the experimental diets.

Item	Gestation diet
Ingredient (%)	
Corn	65.0
Soybean meal	18.0
Self-made premix[a]	10.0
Wheat bran	7.0
Nutrition level (%)	
Dry matter	87.09
Crude protein	19.22
Ether extract	3.75
Crude fiber	6.81
Ash	9.84

[a] Supplied per kilogram of diet: vitamin A, 50,000 IU; vitamin D3, 15,000 IU; vitamin E, 200 IU; vitamin K3, 8 mg; vitamin B1, 10 mg; riboflavin, 40 mg; vitamin B6, 12 mg; nicotinic acid, 200 mg; folic acid, 6 mg; pantothenic acid, 100 mg; sodium chloride, 3.0–6.0%; choline chloride 2000 mg; Fe, 1200 mg as ferrous sulfate; Cu, 200 mg as copper sulfate; Mn, 120 mg as manganese sulfate; Zn, 800 mg as zinc sulfate; I, 1.4 mg as potassium iodide; Se, 1.0 mg as sodium selenite.

2.4. Thermal measurements

In order to test the degree of HS on sows, temperature-humidity index (THI) was calculated according to the formula provided by the previous study (Wegner et al., 2016): THI = [(1.8 *T) + 32]-[(0.55 *(RH/100)]* [((1.8 *T) + 32)-58]. Where T is the ambient temperature in °C and RH is the relative humidity in %. Average hourly THI values were calculated based on daily measurements. After one-week acclimation, thermal response measurements (including rectal temperature, respiration rate, and surface temperature) were taken 4 times each day at 0800, 1200, 1600, and 2000 h during the 2nd week of trial. The data presented in each time point represents the average of the seven-days measures. Rectal temperature was measured with a clinical thermometer (model CRW-12; Yuyue equipment & supply Co., Ltd, Jiangsu, China). Respiration rate was calculated by counting breath per minute for 1 min duration. Movement of the side of the sows was used for this determination. The surface temperature was taken by using an infrared thermographic camera (model TiS40, Fluke Corporation, Everett, USA). The measurement areas were determined according to the previous study (Malmkvist et al., 2012), which were the eye region, snout, and udder. All thermographic pictures were analyzed using Fluke Connect SmartView software, reporting the mean surface temperature (°C) of the selected areas.

2.5. Farrowing data and piglets' BW measurements

Gestation length and duration of parturition were recorded. The

duration of parturition was calculated as the time from first to the last piglet born. Total born, born alive, and litter BW were recorded as the litter data at birth. Piglets were weighed at birth and underwent routine processing procedures (ear notching, tail docking, castration, and supplemental iron injection) in three days after farrowing. Piglets were weighted individually again at d 10 and at weaning.

2.6. Blood and urine sampling

A week before farrowing (the 107 d of gestation), fasting blood samples were collected from sows using jugular venipuncture beginning at 0900 h. A 10 mL blood collection tube with heparin sodium or gel & clot activator (Kangjie equipment & supply Co., Ltd, Jiangsu, China) was used for plasma or serum collection. The blood samples were centrifuged for 15 min at 4 °C and 3000 rpm. The supernatant (plasma or serum) samples were collected and frozen at -20 °C. Urine samples were collected in 2 mL Cryogenic Vials at 0600 h and immediately stored at -80 °C until further analysis.

2.7. Hormone and metabolite assays

Serum ACTH, cortisol, ghrelin, IGF-1, glucagon, insulin, creatinine, BUN, and plasma NEFA levels were determined by commercial ELISA kit according to their instructions (Fangcheng Beijing Technology Co. Ltd, Beijing, China). Assay sensitivities were above 1.6 ng/L, 3.0 μg/L, 160 ng/L, 10 μg/L, 5.0 ng/L, 1.5 mIU/L, 5.0 μmol/L, 0.3 mmol/L and 20 μmol/L, respectively. The intra- and inter-assay coefficient of variations were 9% and 15%, respectively. Plasma glucose, cholesterol and triglyceride concentrations were measured with an automatic biochemical analyzer (Beckman coulter, AU2700) using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China).

2.8. Sample preparation and GC-MS analysis

Urine samples were thawed at 4 °C and centrifuged at 10,000 rpm for 5 min. The supernatant (200 μL) was transferred to a 2 mL Eppendorf tube along with 30 μL urease incubating at 37 °C for 90 min to decompose and remove excess urea present in it. Then 1700 μL methanol (containing 2-Chloro-L-phenylalanine (0.2 mg/mL) and Heptadecanoic acid (0.2 mg/mL) as internal quantitative standard) was added into urinary mixture. The solution was vigorously extracted for 5 min and was centrifuged at 12,000 × *g* at 4 °C for 10 min. The supernatant (1.5 mL) was transferred into another 2 mL centrifuge tube. The samples were dried by vacuum concentration. Then, 60 μL of a 15 mg/mL solution of methoxyamine in pyridine was added into the dried extract, and the mixture was vortexed for 30 s and reacted for 120 min at 37 °C. The methoximation reaction was followed with 60 μL BSTFA reagent (containing 1% FMCS), which was reacted for 90 min at 37 °C and then centrifuged at 12,000 rpm and 4 °C for 10 min. Finally, the supernatant was transferred to a sample bottle for GC-MS analysis (Agilent 7890 A/5975 C, Agilent Technologies, Santa Clara, CA, USA).

The derivatized sample (1.0 μL) was immediately injected by an autosampler into an Agilent 7890 A GC system coupled with an HP-5MS capillary column (5% phenyl/95% methylpolysiloxane, 30 m × 250 μm i.d., 0.25 μm film thickness; Agilent J & W Scientific, Folsom, CA, USA). Helium was used as the carrier gas at a constant flow of 1.0 mL/min through the column. The injection temperature was 280 °C, and the transfer line temperature and ion source temperature were set to 150 °C and 230 °C, respectively. The temperature ramp program was as follows: an initial temperature of 60 °C for 2 min, which was increased at 10 °C/min to 300 °C and held at 300 °C for 5 min. Mass spectrometry was performed using the full-scan method over the range from 35 to 750 *m/z*.

2.9. GC-MS data processing and differential metabolites identification

After the raw data was collected, identification of the compounds was achieved by comparison of the mass spectrum and retention indices of all the detected compounds with their reference standards and database in the National Institute of Standards and Technology Library (http://srdata.nist.gov/gateway/) and Wiley Chemical structure Library (Oberacher et al., 2013). The relative quantitative peak areas of each detected peak were normalized to [$^{13}C_2$]-myristic acid, the stable isotope IS, and the data was arranged on a two-dimensional matrix consisting of arbitrary sample names (observations) and peak area (variables). The multivariate statistical analysis was conducted with the SIMCA-P + version 13.0 software package (Umetrics, Umea, Sweden). The acquired GC/MS data was processed with orthogonal-partial least squares projection to latent structures and discriminant analysis (OPLS–DA). The metabolites with variable importance projection (VIP) values of 1.0 and P-values of 0.05 (threshold) were considered as metabolites that could discriminate between two groups. The impact of HS on metabolic pathways and metabolite set enrichment analysis were evaluated based on an online tool (http://www.metaboanalyst.ca/faces/ModuleView.xhtml) (Chong et al., 2018).

2.10. Statistical analysis

Normal distribution of the data was confirmed before the significance test. Student's *t*-test or Mann-Whitney *U* test (SPSS version 24, IBM Inc Chicago, IL, USA) was used to analyze the effect of HS on eating efficiency, farrowing and litter data and blood biochemical parameters (metabolites and hormones). Moreover, two-way ANOVA (SPSS version 24, IBM Inc Chicago, IL, USA) was used to analyze the effect of HS and time on thermal responses (including RT, ST, and RR) and duration of eating, considering the HS on those parameters as the main effect. Additionally, One-way ANOVA (SPSS version 24, IBM Inc Chicago, IL, USA) was used to compare the changed surface temperature among three different regions. Data were expressed as mean ± SEM or median, and differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Environmental conditions and thermal responses

Figs. 1A and 1B present the hourly mean temperatures and THI values for the TN and HS group during the experimental period. The Environmental Chambers were programmed to achieve daily ambient temperature ranging from 18 °C to 22 °C for TN and 28–32 °C for HS (Fig. 1A). The hourly average THI in the TN group were all in the comfortable range (THI < 74), whereas those in the HS group exceeded 74, ranging from 75 to 80, indicating there was a mild HS in the HS group (Fig. 1B) (Wegner et al., 2016). In particular, the THI values were much higher from 1300 h to 1500 h in the HS group, which were 79.31 ± 0.27, 79.78 ± 0.29 and 79.23 ± 0.28, respectively (Fig. 1B).

The rectal temperature of pregnant sows was higher in the HS group at each time point compared with that in the TN group (Fig. 2A; $P < 0.0001$). The increase in rectal temperature caused by HS was most pronounced at 1600 h (Fig. 2A; 38.39 vs. 38.94 ± 0.09 °C). Similarly, HS elevated respiration rates of pregnant sows at each time point (Fig. 2B; $P < 0.0001$). From 0800 h to 2000 h, average respiration rates between the two groups were differed by 77.9 bpm, 79.5 bpm, 90.6 bpm, and 85.2 bpm, respectively (Fig. 2B). At any time-point, the surface temperature of pregnant sows was higher in the HS group when compared with TN group, regardless of body region measured (Fig. 2C; Fig. 2D; Fig. 2E; $P < 0.0001$). The increase in average surface temperature caused by HS was most remarkable at the snout region (Fig. 2F; $P < 0.001$). Additionally, there was also a time effect

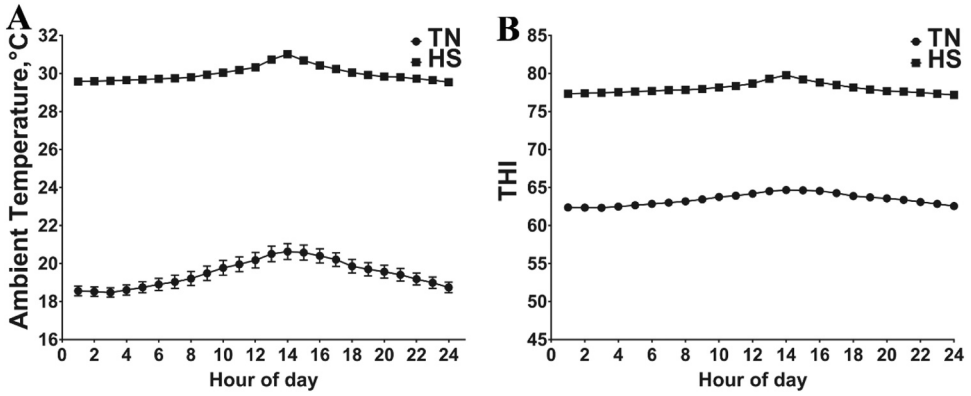

Fig. 1. Hourly variations in ambient temperature (°C) and Temperature-Humidity Index (THI) in the thermoneutral (TN) and the heat stress (HS) chambers of the trial. THI was calculated on the basis of ambient temperature and relative humidity. (A) Hourly averaged ambient temperature, (B) THI values.

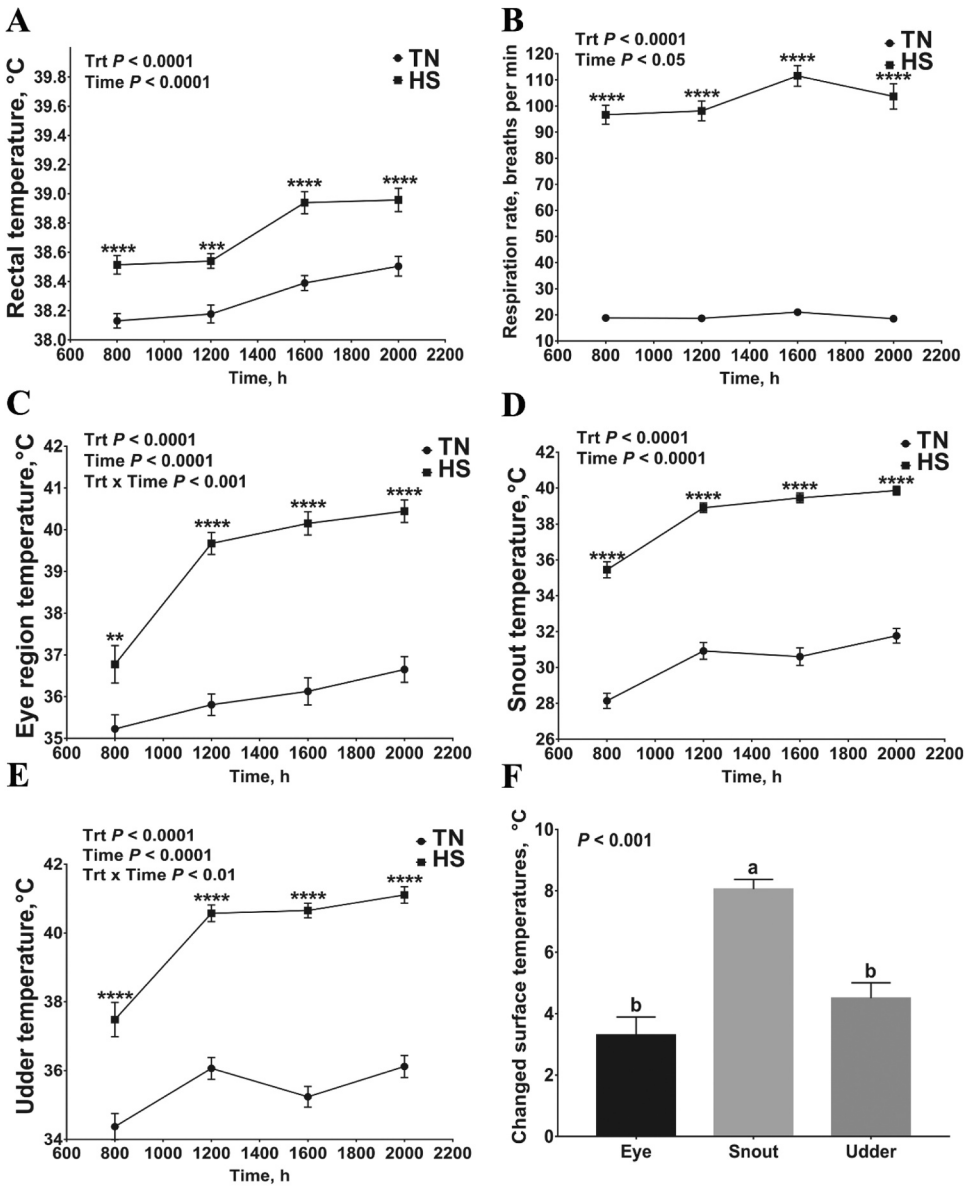

Fig. 2. Heat stress during late gestation increased rectal temperature (°C), respiration rate (Breaths/Min) and the surface temperature (°C) of pregnant sows at different time points. (A) rectal temperature, (B) respiration rate, (C) eye region temperature, (D) snout temperature, (E) udder temperature, (F) changed surface temperatures in the three regions. The changed surface temperature was calculated as the difference of TN and HS groups by an average of four time points. The data showed in this figure represent the average of the seven-days measures. Bars represent means ± SEM, Asterisks (**, ***, ****) means the significant difference between TN and HS group ($P < 0.01$, $P < 0.001$, $P < 0.0001$), and (^{a,b}) means without a common letter differ ($P < 0.001$).

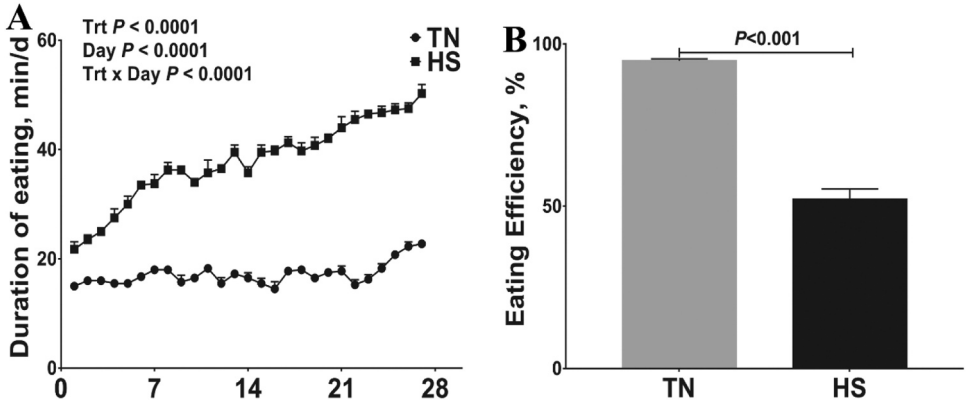

Fig. 3. Heat stress during late gestation reduced feed intake measurements of pregnant sows. (A) Duration of eating. Treatment, time, and treatment by time interaction were all significant ($P < 0.0001$) for the duration of eating. (B) Eating efficiency. Bars represent means ± SEM.

on the rectal temperature, respiration rates, and surface temperature that increased from 0800 h to 2000 h (Fig. 2A, Fig. 2C, Fig. 2D, Fig. 2E, $P < 0.0001$; Fig. 2B, $P < 0.05$).

3.2. Feed intake measurements

Since pregnant sows were limit-fed during late gestation with an average of 3.0 ± 0.1 kg/d feed intake, the duration of eating and eating efficiency were used to assess the feeding behavior. It was observed that both treatment and time factors affected the duration of eating throughout the experiment (Fig. 3A; $P < 0.0001$), the HS sows had a longer time for feed intake than did TN sows, and the time increased gradually during late gestation for both groups. There was also an interaction between treatment and time regarding the duration of eating during late gestation (Fig. 3A; $P < 0.0001$). Moreover, the eating efficiency was reduced by nearly 50% in the HS group (Fig. 3B; 94.78% for the TN group vs. 52.16% for the HS group), indicating a much more intermittent eating behavior in the HS group.

3.3. Farrowing and litter data

The duration of parturition (Table 2; 5.2 h for the TN group vs. 6.5 h for the HS group) was prolonged in the HS group during the delivery (Table 2; $P < 0.05$). Gestation length, total born, born alive and litter BW at processing were not affected by HS (Table 2; $P > 0.10$). Piglet BW at born was not affected by maternal HS. However, piglet BW at d 10 and weaning were reduced by nearly 18% and 17% respectively due to maternal HS (Table 2; $P < 0.001$). Moreover, piglet daily creep feed consumption per litter was doubled by maternal HS before weaning (Table 2; $P < 0.01$).

Table 2
Farrowing and litter data for TN and HS sows in late gestation.

Item	Treatments		*P*-value[1]
	TN	HS	
n	5	6	–
Gestation length, d	113.80 ± 1.24	115.00 ± 1.00	NS
Duration of parturition, h	5.20 ± 0.26	6.50 ± 0.43	< 0.05
Litters at birth			
Total born, n	14.4 ± 1.78	15.00 ± 0.58	NS
Born alive, n	12.8 ± 1.99	13.00 ± 0.71	NS
Litter BW, kg	17.50 ± 1.95	17.48 ± 0.98	NS
Piglets born/H	2.85 ± 0.47	2.37 ± 0.21	NS
Piglet BW			
BW at d 0, kg	1.37 ± 0.04	1.33 ± 0.03	NS
BW at d 10, kg	3.47 ± 0.12	2.86 ± 0.10	< 0.001
BW at weaning, kg	6.92 ± 0.26	5.80 ± 0.19	< 0.001
Piglet daily creep feed consumption g/litter	51.70 ± 11.24	103.20 ± 11.80	< 0.01

[1] NS = not significant ($P > 0.10$).

Table 3
Hormones and metabolites for TN and HS sows in late gestation.

Item	Treatments		*P*-value[1]
	TN	HS	
n	5	6	–
Serum ACTH, ng/L	58.79 ± 1.03	69.01 ± 2.77	< 0.05
Serum Cortisol, ng/mL	51.20 ± 0.69	65.36 ± 1.43	< 0.001
Serum Ghrelin, ng/L	3192.78 ± 80.69	2639.08 ± 133.10	< 0.05
Serum IGF-1, μg/L	182.45 ± 4.44	156.08 ± 9.19	< 0.05
Plasma Glucose, mmol/L	7.42 ± 0.48	7.05 ± 0.48	NS
Serum glucagon, ng/L	85.84 ± 3.02	83.05 ± 2.38	NS
Serum Insulin, mIU/L	27.93 ± 0.91	36.11 ± 1.05	< 0.05
Plasma Cholesterol, mmol/L	0.19 ± 0.02	0.21 ± 0.02	NS
Plasma Triglyceride, mmol/L	0.79 ± 0.11	0.68 ± 0.07	NS
Plasma NEFA, μmol/L	328.30 ± 7.91	481.21 ± 8.87	< 0.001
Serum Creatinine, μmol/L	94.51 ± 2.04	124.30 ± 2.89	< 0.001
Serum BUN, mmol/L	4.13 ± 0.15	5.56 ± 0.13	< 0.001

[1] NS = not significant ($P > 0.10$).

3.4. Blood biochemical parameters

HS significantly impacted many serological and hematological indices in pregnant sows during late gestation (Table 3). As endocrine stress indicators, serum concentrations of ACTH ($P < 0.05$) and cortisol ($P < 0.001$) were elevated in HS group. Ghrelin and IGF-1, two hormones related to feed intake and growth, their serum concentrations were decreased in HS group ($P < 0.05$). For carbohydrate metabolism, there was no effect of HS on plasma glucose and serum glucagon concentrations, whereas serum insulin concentration was increased in HS group ($P < 0.05$). For lipid metabolism, plasma cholesterol and triglyceride concentrations were not affected by HS. However, plasma NEFA concentration was elevated in HS group ($P < 0.001$). For protein metabolism, serum creatinine ($P < 0.001$) and BUN concentrations ($P < 0.001$) were greater in the HS group.

3.5. Metabolomics profiling in urine

HS had a great impact on metabolite profiles in the urine of pregnant sow. GC/MS-based measurement identified 77 metabolites in urine. These metabolites can be divided into seven major groups, namely, organic acids, amino acids, carbohydrates, polyols, fatty acids, amines, and phosphoric acids, on the basis of the characteristic of each chemical. OPLS-DA model showed that there was a clear separation in

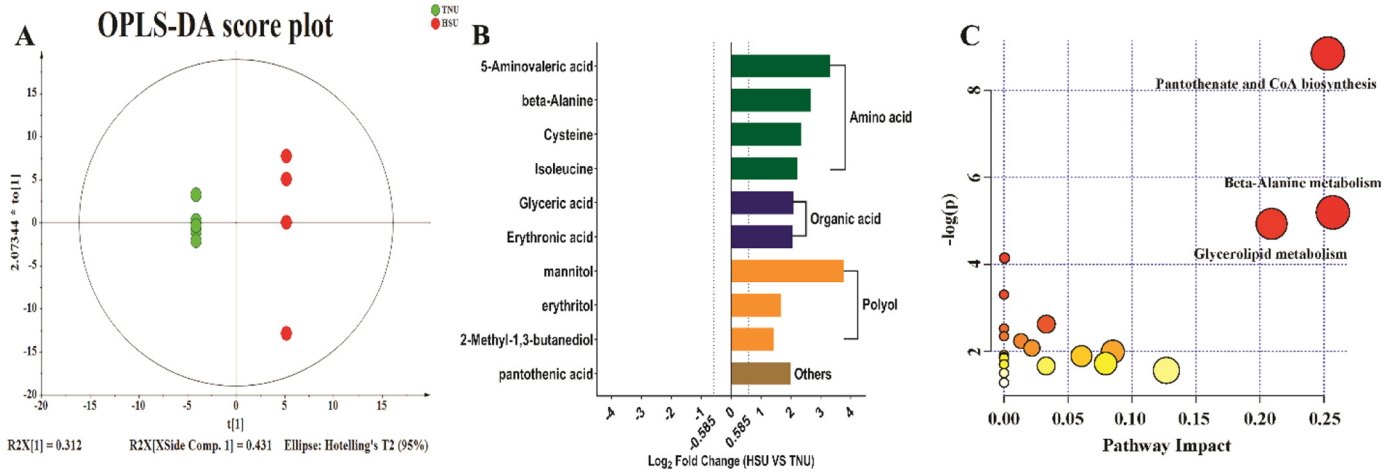

Fig. 4. Effect of HS on metabolomic profiles for sows in late gestation. (A) Orthogonal-partial least squares projection to latent structures and discriminant analysis (OPLS-DA) based on the urinary compound data. The OPLS-DA score plots discriminating between the urine of sows treated TN (green, left) and HS (red, right) condition during late gestation [predictive ability parameter (Q^2) (cum) = 0.789, goodness-of-fit parameter (R^2) (X) = 0.987]; (B) Significant compounds. Metabolites accountable for class discrimination with *VIP* > 1, *log₂ fold change* > 0.585, and $P < 0.05$ were listed; (C) Metabolome view map of the differential metabolites (*VIP* > 1) identified in the urine from the sows treated thermoneutral (TN) and heat stress (HS) condition during late gestation. The x-axis represents the pathway impact and the y-axis represents the pathway enrichment. The node color is based on its *P*-value, and the node radius is determined based on the pathway impact values. Larger sizes and darker colors represent higher pathway enrichment and higher pathway impact values, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

metabolites between the HS and TN group, which suggested that urine metabolic profiles of two groups were significantly different under HS (Fig. 4A). For urinary metabolites, HS elevated (*VIP* > 1, *log₂ fold change* > 0.585, and $P < 0.05$) the relative concentrations of 5-aminovaleric acid, β-alanine, cysteine, isoleucine, glyceric acid, erythronic acid, mannitol, erythritol, 2-methyl-1,3-butanediol, and pantothenic acid (Fig. 4B). Further metabolic pathway enrichment analysis indicated that these ten metabolites mainly affected the pantothenate and CoA biosynthesis, β-alanine metabolism, and glycerolipid metabolism of pregnant sows (Fig. 4C; $P < 0.05$).

4. Discussion

In the present study, we investigated thermoregulation, productive performance and especially metabolic responses in the primiparous sows exposed to HS during late gestation. Unlike the previous study, we used constant HS during late gestation and GC-MS analysis for metabolite profiles (Williams et al., 2013). Our results showed that controlled constant HS during late gestation elevated thermal responses, reduced productive performance, and more importantly accelerated lipid and protein catabolism of first-parity pregnant sows.

Firstly, a constant ambient temperature (20 °C) was set to achieve daily ambient temperature ranging from 18 °C to 22 °C for TN group since the optimum temperature is 18 °C with a desirable limit of 10–27 °C for pregnant sows (Myer, 2001). The HS group temperature was set to 30 °C to mimic the ambient temperature in the gestation barn in the summer of Jiangsu Province. The external ambient temperature in Jiangsu Province in July and August is usually around 35 °C, the fan and pad evaporative cooling system is used to decrease the ambient temperature by 5–7 °C inside the gestation barn (Lucy and Safranski, 2017). That is why we set the HS group temperature to 30 °C. Furthermore, THI was used as an index to assess the level of environment-induced heat stress in humans and farm animals (Aggarwal and Upadhyay, 2012). Based on Wegner's study for sows, THI below 74 is a comfort zone, 75–78 as mild stress, 79–83 as moderate stress, and above 84 as severe stress (Wegner et al., 2016). In our study, the THI values were all ranging from 60 to 65 in the TN group, whereas 75–80 in the HS group, especially higher than 79 from 1300 h to 1500 h. These results indicated that sows in the HS group were under mild even moderate stress while sows in the TN group were in a comfort zone. Moreover, the THI values also reflected the successful establishment of our HS model for pregnant sows.

Rectal temperature, respiration rate, and surface temperature were measured during the second week of the four-week heating intervention. Our results showed that HS during late gestation increased sows' rectal temperature, respiration rate, and surface temperature. Data from previous studies was in agreement with our result that HS sows had a higher rectal temperature during gestation (Omtvedt et al., 1971; Williams et al., 2013), indicating that sows were not able to fully control body temperature at the high temperature we applied at HS group. Interestingly, the effect of HS on rectal temperature during late gestation was greater in our study (approximately 0.5 °C) than that in the previous one (approximately 0.1 °C) (Williams et al., 2013). This perhaps because the constant HS we used in our study made sows more stressed than the cyclic way. Moreover, respiration rate we observed was also much greater than other studies in the HS group (Liao and Veum, 1994; Williams et al., 2013). For instance, Williams et al. (2013) found a 2-fold increase in respiration rate in late gestation sows exposed to HS, whereas what we observed was about 5-fold. This severe increase of respiration rate should also be related to the constant HS we applied. The sows in our study had suffered severe HS during late gestation, and they attempted to cool themselves through an increase in respiration rate (Seibert et al., 2018). Additionally, the skin temperature of three different body regions (eye, snout, udder) were all increased in the HS group since blood was shunted from internal organs to the peripheral circulation. Among these three body regions, snout seemed to be more susceptible to HS, implying its good capacity to dissipate heat in the surface. As a whole, the increase of respiration rate and surface temperature in this study indicated pregnant sows augmented their thermoregulatory behavior during late gestational HS, however, it still cannot neutralize the HS effect as a proof of their increased rectal temperature. It is worth noting that these thermal responses may be less extreme at a later time point due to acclimation.

Feed intake measurements, as well as farrowing and litter data, were monitored to estimate sows' productive performance. We used the duration of eating firstly to assess the feeding behavior. Our results showed that HS sows had a longer duration of eating than did TN sows, and it increased gradually with time during late gestation. In addition, the eating efficiency decreased by approximately 50% from TN to HS group, indicating there was a much more intermittent time (water

consumption and lying) when sows were eating in the HS group. The decreased appetite caused by HS and farrowing should be the possible reason for constantly increased feeding duration, and HS may play a more important role. Besides, the significant decrease of serum ghrelin in HS sows may confirm this effect.

For farrowing and litter data, late gestational HS did not shorten the gestation length in our study, but we observed a prolonged duration of parturition that was consistent with previous reports (Muns et al., 2014; Oliviero et al., 2008). The prolonged duration of parturition indicated the increased risk of parturition for sows in the HS group. Moreover, there was no effect of late gestational HS on total born, born alive, or stillborn, which agreed with a previous report by Williams et al. (2013). This perhaps because the HS we used only presented in the last month of pregnancy. The farrowing data related to the embryonic loss was not affected during this period. Intriguingly, there was no effect of late gestational HS on piglet BW at born, but it reduced piglet BW at weaning by approximately 1.1 kg. This is about 17% decrease compared with the piglet BW in the TN group, even though the sows were all moved to the thermoneutral environment after farrowing. Some studies have observed lactational HS-sows were associated with reduced litter growth at weaning (Quiniou and Noblet, 1999; Renaudeau and Noblet, 2001; Silva et al., 2009). In our study, the compromised lactation induced by HS during late gestation should be the probable reason for the reduced piglet BW at weaning. Our speculation was similar to what is observed in the dairy cattle. Tao and his colleagues reported that the HS during late gestation impaired mammary growth before parturition and decreased milk production in the subsequent lactation (Tao and Dahl, 2013). Additionally, elevated piglets' creep feed consumption in the HS group, indicating the deficiency of lactation yield, was another evidence for the compromised lactation. Herein, our litter data suggests that late gestational HS may have a negative effect on the following lactation period in sows.

In addition to the detection of sows' thermal responses and productive performance to the HS during late gestation, we also focused on their metabolic responses by blood biochemical parameters and urinary GC-MS analysis. Firstly, we found the serum concentrations of ACTH and cortisol both increased in the HS group, indicating that the hypothalamic-pituitary-adrenal (HPA) axis got activated in response to HS in our study as well (Malmkvist et al., 2009). Also, cortisol has been reported to act as vasodilator to help heat loss, and have a stimulatory by blood biochemical parameters and urinergy (through the gluconeogenesis) to the animals by blood biochemical parameters and urine (Cunningham and Klein, 2007). For lipid metabolism in our study, HS increased plasma NEFA concentration, which is supported by the previous study that NEFA was elevated promptly in HS sows from late gestation to early lactation (Lucy and Safranski, 2017). Intriguingly, it has been proved that HS increased lipid retention with typically reduced NEFA level in various animal species including pigs (Ross et al., 2017). These two statements seem to conflict, but actually inclusive pregnancy, a unique physiological status, could make a difference. Animals are hard to get enough nutrients to meet fetal growth, milk preparation and maintenance costs during late gestation, which easily brings them into the negative energy balance (Drackley, 1999). Once it happens, somatotropin will stimulate adipose tissue to release NEFA for accentuating the lipolytic response to β-adrenergic signals and inhibiting insulin-mediated lipogenesis and glucose utilization (Bauman and Vernon, 1993; Luo and Liu, 2016). Moreover, our urinary GC-MS analysis indicated that both glyceric acid and glycerolipid pathway enhanced in the HS group. Glyceric acid was converted from glycerol via glycolysis to supply energy. Hence, late gestational HS promoted lipid catabolism due to not only the elevated NEFA level but also the enhanced glycerolipid pathway. For protein metabolism, HS raised serum concentrations of BUN and creatinine in our study. BUN originates from increased protein catabolism as gluconeogenic substrates in a variety of heat-stressed species (Baumgard and Rhoads Jr, 2013; Min et al., 2017; Pearce et al., 2013). Creatinine, the degradation product of creatine phosphate, plays an important role in energy balance when animals have high energy requirements (Liao et al., 2018). Furthermore, the urine levels of glucogenic amino acids, such as 5-aminovaleric acid, β-alanine, cysteine, and isoleucine, which could generate pyruvate, succinic acid, and acetoacetic acid, were higher in the HS group compared with TN group. Therefore, protein catabolism and amino acids mobilization were elevated for subsequent energy supply during HS. This protein catabolism might result in reduced milk protein synthesis (Cowley et al., 2015), which is responsible for the reduced body weight of weaned piglets in maternal HS group in our study. In addition, pantothenate and CoA biosynthesis pathway was also affected in our study. CoA is an essential cofactor for pyruvate to enter the tricarboxylic acid cycle (TCA cycle) as acetyl-CoA. The enhanced pathway was also evidence for the increased lipid and protein catabolism discussed above. For carbohydrate metabolism, we found that no effect of HS on plasma glucose and serum glucagon concentrations, whereas serum insulin concentration was increased in the HS group. This result was in agreement with several previous studies stated heat-stressed animals were often hyperinsulinemic even though the well-documented reduction was in feed intake and body weight (Baumgard and Rhoads Jr, 2013; O'Brien et al., 2010; Pearce et al., 2013; Wheelock et al., 2010). Reasons for hyperinsulinemia during HS are not clearly understood till now, but recent studies suggest that HS-induced elevated circulating LPS is likely a large contributor to the overall improved insulin secretion (Baumgard et al., 2016; Sanz Fernandez et al., 2015).

Given the alterations of metabolites in the urine, we should not ignore the crucial role of renal function that may impact their levels. Leon and her colleagues well reported that renal failure is a common finding in heat stroke of humans, which induced renal tubular necrosis in the straight tubules of the kidney lower cortex (Leon and Helwig, 2010; Sawka et al., 2011). Serum BUN and creatinine, significantly elevated in our study, are two traditional clinical biomarkers of the renal function. Kidneys are responsible for removal of BUN and creatinine from circulation and their elevated levels might be due to a HS-mediated reduction in renal filtration. In this context, the increased concentrations of all differential metabolites in the urine may be associated with the compromised renal function, specifically resorption capacity.

5. Conclusion

In this study, controlled HS during late gestation induced thermal, productive, and metabolic responses in sows mainly through activating HPA axis. In summary, HS elevated rectal temperature, respiration rate and surface temperature in sows. Moreover, HS decreased eating efficiency, increased duration of eating and parturition of sows, as well as elevated piglets daily creep feed consumption and reduced piglets BW at d 10 and weaning. The results of metabolic responses suggest HS exacerbated the negative energy balance and altered the nutrition partition for energy supply during late gestation. Specifically, protein (amino acids) and lipid (NEFA and glycerol) catabolism were enhanced in HS conditions. From the above description, the evaporative cooling system alone (i.e. the temperature used in HS group) is not enough to help pregnant sows overcome high temperature in summer of Jiangsu Province, and further investigation should be focused on the effect of additional nutritional interventions for ameliorating HS in pregnant sows in summer.

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Declaration of interests

The authors declared that there are no conflicts of interest to this work.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2019.01.011.

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