Energy partition patterns and plasma metabolomics profiles of modern growing pigs under different ambient temperatures: modelling and integrative analysis

Shuai Zhang

China Agricultural University <https://orcid.org/0000-0001-5435-2640>

Hang Gao

China Agricultural University

Xiongkun Yuan

China Agricultural University

Junjun Wang

China Agricultural University

Jianjun Zang (✉ zangjj@cau.edu.cn)

China Agricultural University

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Abstract

Background

Most of the studies focusing on energy partition patterns of growing pigs and the related mechanisms under different ambient temperatures were carried out during 1970s to early 2000s. With the rapid developments in pig breeding, research updates on such topics concerning modern growing pigs have been absent in the last decade. This study developed mathematical models to predict the energy partition patterns of modern growing pigs with different BWs at gradient-ambient temperatures, and investigated the underlying changes in plasma under such conditions based on the integrative analysis of indirect calorimetry and non-target metabolomics profiling.

Methods

In trial 1, 36 barrows with initial BW of 26.4 ± 1.9 kg were allotted to a 6×6 Latin Square Design with ambient temperatures set as 18 °C, 21 °C, 23 °C, 27 °C, 30 °C, and 32 °C. In trial 2, 24 barrows with initial BW of 64.2 ± 3.1 kg were allotted to a 4×6 Youden Square Design with ambient temperatures set as 18 °C, 23 °C, 27 °C, and 32 °C. Each pig was kept in individual metabolic crate and consumed feed *ad libitum*, then transferred into the respiration chamber for 5-day indirect calorimetry assay and 1-day fasting. The energy and nitrogen balance as well as energy utilization efficiency were determined, and mathematical models were developed to predict the voluntary feed intake (VFI), metabolizable energy intake (ME_i) and energy retention as protein (RE_p) and lipid (RE_L) using BW and ambient temperature (T) as predictors. Moreover, plasma samples were collected from pigs at 25 kg under different temperatures, and the levels of hormone and metabolites in plasma were determined using biochemical and the metabolomics approaches, respectively.

Results

As the ambient temperature increased from 18 °C to 32 °C, the VFI, ME_i , N intake and retention, total heat production (THP) and RE_p of growing pigs at 25 kg all linearly decreased ($P < 0.05$). There were interaction effects between BW and T on VFI, ME_i , N balance, adjusted THP, RE_p and RE_L ($P < 0.05$). Parallel curves were observed between the VFI model developed in the current study and that built previously, but our ME_i model showed discrepancy from the previous model especially at high temperatures. The models simulated for RE_p and RE_L are: RE_p (kJ/kg $BW^{0.6}/d$) = $-233.38 - 67.28 \times BW^{0.6} + 3.16 \times (BW^{0.6})^2 + 39.58 \times T - 0.76 \times T^2 - 0.26 \times BW^{0.6} \times T + 0.26 \times ME_i$, and RE_L (kJ/kg $BW^{0.6}/d$) = $-465.10 + 8.10 \times BW^{0.6} - 1.03 \times (BW^{0.6})^2 - 53.73 \times T + 1.34 \times T^2 + 0.67 \times BW^{0.6} \times T + 0.69 \times ME_i$, respectively. The cortisol and thyroid hormone levels in plasma linearly decreased as the ambient temperature increased from 18 °C to 32 °C, and 13 compounds were identified through metabolomics analysis, including up-regulated metabolites involved in fatty acid metabolism such as adrenic acid and down-regulated metabolites involved in amino acid metabolism such as spermidine at 32 °C.

Conclusion

These results suggested that pigs at heavier body weight were more sensitive to high temperatures on energy intake and partition, and pigs with modern genotypes could be more sensitive to heat stress than pigs with older genotypes. Most of the identified metabolites altered under high ambient temperatures are associated with suppressed fatty acid oxidation and elevated lipogenesis and protein degradation.

Background

Moderate ambient temperature in house is the prerequisite for animals to maintain health and performance. Especially in the modern large-scaled and high-efficient livestock production system, high or low temperature is one of the major stressors that animals face. Under thermoneutral zone, the dietary energy intake is used by animals to meet the requirements of maintenance, physiological activity, and production (e.g. growth, reproduction) [1]. When the ambient temperature is below the lower critical temperature (LCT) of the thermoneutral zone, heat production (HP) increases in warm-blooded animals such as pigs to meet the additional energy requirement of thermoregulation, with more feed are consumed to maintain the growth performance [2]. On the other hand, the feed intake, HP, and energy retention (RE) of pigs will decrease when the ambient temperature is above the upper critical temperature (UCT) of the thermoneutral zone, along with the reduced locomotion [3]. Those threshold temperatures vary depending on animal-related factors such as genotype, body weight (BW), and physiological stage [4].

The research on effects of ambient temperature on patterns of physiological changes in livestock started on 1940s, with the application of artificial climate warehouse to mimic the environment in animal houses. In pigs, many studies were conducted from 1970s to early 2000s focusing on energy partition patterns under different temperatures using the climate-controlled open-circuit respiratory chamber [1–3, 5–9]. With the development of animal breeding, modern pigs have great progress in productivity (e.g. quick weight gain and higher reproductivity), but their bodies become more sensitive to adverse environment such as changes in ambient temperatures [10]. Therefore, researches were carried out in recent years concerning about the alternations and alleviation strategies of modern pigs under heat or cold stress. However, there is few updates on patterns of energy balances in modern pigs at different ambient temperatures especially in the last decade.

Indirect calorimetry is the traditional non-invasion approach to determine the HP of the subjects, and has been widely used in livestock to monitor and model the energy partition patterns together with the analysis of feed intake and urine and feces outputs [11]. Moreover, the metabolic changes underlying the energy reactions has received more attention in recent years, which could directly reflect the regulation and adaptation of the animals to the surrounding conditions, and may provide novel diagnostic tools or biomarkers with high sensitivity towards the adverse effects [4]. The non-targeting metabolomics is an ideal approach to analyze the comprehensive changes in metabolites of the subjects, and has never been used to explore the relationships between metabolites alternation and the patterns of energy expenditure or substrate oxidation of growing pigs under various ambient temperatures.

Therefore, the objectives of this study were to determine and establish models for the energy partition patterns of modern growing pigs with different BWs at gradient-ambient temperatures, and to identify the underlying changes in energy metabolism under such conditions based on the integrative analysis of indirect calorimetry and non-target plasma metabolomics profiling.

Methods

The Institutional Animal Care and Use Committee of China Agricultural University (Beijing, China) approved all protocols used in this experiment.

Equipment for indirect calorimetry

Six open-circuit respiration chambers ($1.4 \times 2.7 \times 2.1 \text{ m}^3$) located in the FengNing Swine Research Unit of China Agricultural University (Academician Workstation in Chengdejiuyun Agricultural & Livestock Co., Ltd) were used to conduct the indirect calorimetry, which were described in details in previous studies [12, 13] with the exception of temperature settings. The relative humidity was controlled at 70–75%, and the air velocity was controlled at 0.1 m/s. Oxygen content was measured with a paramagnetic differential analyzer (Oxymat 6E, Siemens, Munich, Germany), whereas CO_2 and CH_4 contents were measured with infrared analyzers (Ultramat 6E, Siemens). The analyzers had a range of 19.5–21.0% for O_2 , 0–1% for CO_2 , and 0–0.1% for CH_4 , with a sensitivity of 0.2%. Gas concentrations in each chamber were measured at 5-min intervals. The ethanol combustion experiment was used to check the accuracy of the chamber in measuring gaseous exchange.

Animals, diets and experiment design

Two animal trials were conducted in this study. In Trial 1, 36 healthy growing barrows (Duroc $\times$ Large White $\times$ Landrace) with initial BW of $26.4 \pm 1.9 \text{ kg}$ were allotted to a 6×6 Latin Square Design in 6 consecutive periods. In each period, 6 pigs were moved into 6 open-circuit respiration chambers with ambient temperatures setting as 18 °C, 21 °C, 23 °C, 27 °C, 30 °C, and 32 °C, respectively. In Trial 2, 24 healthy growing barrows (Duroc $\times$ Large White $\times$ Landrace) with initial BW of $64.2 \pm 3.1 \text{ kg}$ were allotted to a 4×6 Youden Square Design in 6 consecutive periods. In each period, 4 pigs were moved into 4 open-circuit respiration chambers (only 4 chambers were available during Trial 2) with ambient temperatures setting as 18 °C, 23 °C, 27 °C, and 32 °C, respectively.

Two diets based on corn-soybean meal were formulated to meet or exceed the nutrient requirements for growing pigs at 25 kg and 65 kg according to the recommendations by NRC [14], and were applied in Trial 1 and 2, respectively. The analyzed chemical compositions of those two diets were shown in Table 1.

Table 1

Ingredient compositions and analyzed chemical components of the complete diets fed to barrows at different growth stages used in Exp.1 and Exp.2 (as-fed basis)

Items	Growth stage	
	25 kg	65 kg
Ingredients, %		
Corn	77.13	80.39
Soybean meal	20.00	17.00
Dicalcium phosphate	0.90	0.80
Limestone	0.75	0.65
Sodium chloride	0.35	0.35
Premix ¹	0.50	0.50
Lysine-HCl	0.21	0.17
DL-Methionine	0.11	0.09
L-Threonine	0.02	0.02
L-Tryptophan	0.03	0.03
Analyzed nutrient levels, %		
Dry matter	89.50	89.60
Gross energy, MJ/kg	16.38	16.67
Crude protein	17.78	16.20
Ether extract	2.89	2.57
Ash	4.72	3.34
¹ Premix supplied the following quantities per kilogram of complete diet for pigs at 25 kg: vitamin A, 6,000 IU; vitamin D ₃ , 1,500 IU; vitamin E, 15 IU; vitamin K ₃ , 1.5 mg; vitamin B ₁₂ , 10 µg; riboflavin, 3.0 mg; pantothenic acid, 9.5 mg; niacin, 18 mg; choline chloride, 350 mg; folacin, 0.4 mg; thiamine 0.9 mg; pyridoxine 1.5 mg; biotin, 25 µg; Mn, 17.5 mg (MnO); Fe, 80 mg (FeSO ₄ ·H ₂ O); Zn, 65 mg (ZnO); Cu, 60 mg (CuSO ₄ ·5H ₂ O); I, 0.3 mg (KI); Se, 0.2 mg (Na ₂ SeO ₃), and supplied the following quantities per kilogram of complete diet for pigs at 65 kg: vitamin A, 5,000 IU; vitamin D ₃ , 1,350 IU; vitamin E, 13.5 IU; vitamin K ₃ , 1.45 mg; vitamin B ₁₂ , 9 µg; riboflavin, 2.7 mg; pantothenic acid, 8.0 mg; niacin, 16 mg; choline chloride, 280 mg; folacin, 0.3 mg; thiamine 0.7 mg; pyridoxine 1.35 mg; biotin, 20 µg; Mn, 15.0 mg (MnO); Fe, 70 mg (FeSO ₄ ·H ₂ O); Zn, 65 mg (ZnO); Cu, 25 mg (CuSO ₄ ·5H ₂ O); I, 0.3 mg (KI); Se, 0.2 mg (Na ₂ SeO ₃).		

The specific experiment procedures were kept the same in both animal trials. In each period, pigs were kept individually in stainless-steel metabolism crates (1.2 × 0.5 × 0.6 m³) equipped with feeders and low-pressure nipple drinkers, and had a 7-d adaption period for crates and diets in the thermoneutral-controlled room (24 ± 1 °C). On day 8, pigs were weighted and moved into the chambers for 5 days to measure the gases exchanges and calculate the total heat production (THP). Based on the feed intake during adaption, feed was supplied twice daily (08:30 and 15:30) *ad libitum* to pigs in chambers, and pigs had free access to water. For each pig, the total feces, urine output and wasted feed were collected twice daily from 08:00 to 08:30, and from 15:00 to 15:30, when the production of CO₂, CH₄ and O₂ consumption were expelled in the calculation of daily HP, to determine the energy intakes of each day. On day 13, pigs were weighted in the morning and deprived of feed and fasted for 24 h, and the total urine output were collected during the fasting period to determine the fasting heat production (FHP), as an estimation of the energy used for maintenance [12]. On the morning of day 14, pigs were moved out the chambers and weighted again.

Sample collection

The daily urine output of each pig was collected into buckets with 50 mL 6 N HCl and sieved thereafter through multilayer gauze into plastic bottles and stored in -20 °C. The 5-day total urine collection and urine collection during the fasting period were quantified for each pig respectively, and a subsample of 50 mL for each pig was saved from the thoroughly mixed 5-day total collection for further analysis. The daily feces output of each pig was collected into plastic bags and immediately stored at -20 °C, then the 5-day total feces collection

was thawed, mixed, and weighted, and a subsample of 350 g for each pig was oven-dried for 72 h at 65 °C to calculate the moisture content. Feed samples and subsamples of dried feces were finely ground through a 1 mm sieve prior to chemical analysis.

Blood samples of pigs in Trial 1 were collected from precaval vein into the heparinized tubes on day 13 morning during fasting, and then were centrifuged (Biofuge22R; Heraeus, Hanau, Germany) at $3,000 \times g$ for 10 min at 4 °C. The supernatants were transferred to storage tubes, frozen in liquid nitrogen, and stored at -80 °C for further assays.

Chemical analysis and calculation

All chemical analysis of samples was conducted in duplicates and repeated if the duplicates differed by more than 5%. The gross energy (GE) in feed, feces, and urine samples was determined using an isoperibol calorimeter (Parr 6400 Calorimeter, Moline, IL, USA) with a standard reference of benzoic acid. For feed samples, the contents of dry matter (DM), crude protein (CP), ether extract (EE), and ash were determined following the methods of AOAC [15]. For fecal samples, the contents of DM and CP were determined. For urine samples, the nitrogen (N) content was measured according to Li et al. [13].

The dry matter intake (DMI) of each pig from day 8 to day 12 was calculated as the product of feed intake and the DM content of the diets. The GE intake was calculated as the product of actual DMI during the 5-day collection period and the GE values of the diets. The digestible energy (DE) intake and metabolizable energy intake (ME_i) was calculated as the difference between GE intake and the energy loss in feces, and the difference between DE intake and the energy loss in urine and methane, respectively. Methane energy was calculated using the methane volume and a conversion factor of 39.4 kJ/L [16].

The average daily THP was calculated according to the following equation based on the gas exchanges (O_2 consumption and productions of CO_2 and CH_4) during day 8 to day 12 recorded at 5-min intervals and then averaged and extrapolated to a 24-h period:

$$HP \text{ (kJ)} = 16.18 \times O_2 \text{ (L)} + 5.02 \times CO_2 \text{ (L)} - 2.17 \times CH_4 \text{ (L)} - 5.99 \times \text{urinary N (g)} \text{ [16].}$$

The FHP was calculated using the same equation, but the 24-h FHP was predicted from the 8-h HP after feed deprivation from 22:00 to 06:00 during the last day of each period, which was then extrapolated to the 24-h period [13]. The RE was calculated as the difference between ME_i and THP, while the RE as protein (RE_p) was calculated as N retention (g/d) $\times$ 6.25 $\times$ 23.86 (kJ/g), and the RE as lipid (RE_l) was calculated as the difference between RE and RE_p . The net energy (NE) intake was calculated as the sum of RE and maintenance energy estimated by FHP [17]. Moreover, the oxidation of carbohydrates (OXCHO) and fat (OXF) were calculated by the method described by Chwalibog et al. according to the following equations [18]:

$$OXCHO \text{ (kJ/d)} = [-2.968 \times O_2 \text{ (L)} + 4.147 \times CO_2 \text{ (L)} - 1.761 \times CH_4 \text{ (L)} - 2.446 \times \text{Urinary N (g)}] \times 17.58.$$

$$OXF \text{ (kJ/d)} = [1.719 \times O_2 \text{ (L)} - 1.719 \times CO_2 \text{ (L)} - 1.719 \times CH_4 \text{ (L)} - 1.963 \times \text{Urinary N (g)}] \times 39.76.$$

The rates of DE:GE, $ME:DE$ and $NE:ME$ were then calculated based on the calculations of GE, DE, ME, and NE intakes. The respiratory quotient (RQ) was calculated as the ration between CO_2 production and O_2 consumption. All the energy balance indexes were present on the metabolic body weight (per kg $BW^{0.60}$) basis.

Hormone and biochemical marker assays in serum

The concentrations of cortisol, insulin, triiodothyronine (T_3) and thyroxine (T_4) in serum were analyzed using radioimmunoassay, while the activities of growth hormone (GH) and glucagon in serum were analyzed using commercial enzyme-linked immunosorbent assay (ELISA) kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's guides. The levels of albumin, glutamic-pyruvic transaminase (ALT), glutamic oxalacetic transaminase (AST), globulin, high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (TC), triglyceride (TG), total protein and urea in serum were determined using the automatic biochemical analyzer (7170, Hitachi Corp., Tokyo, Japan) with corresponding commercial kits (Nanjing Jiancheng Bioengineering Institute) following the manufacturer's guides.

Non-target metabolomics profiling in plasma and data analysis

Plasma samples from pigs kept under the ambient temperatures of 18 °C, 23 °C and 32 °C (defined as low, neutral and high ambient temperature group, respectively) were used for non-target metabolomics profiling assay, followed the procedures described by Liu et al. with some modifications [19].

Briefly, 100 µL plasma samples were added into 400 µL ice-cold extraction mix (methanol: acetonitrile = 1:1, v: v). After vortexing for 10 s, the mixture was centrifuged (Eppendorf, Germany) at $15,000 \times g$ for 10 min at 4 °C to remove protein, and 400 µL supernatant was collected and evaporated to dryness using a vacuum concentrator (Concentrator PLUS, Eppendorf). The resulting dry residues were re-suspended in 200 µL recovery solution (water: methanol = 4:1), vortexed and centrifuged again at $15,000 \times g$ for 10 min at 4 °C. The supernatant was filtered through a 0.22 µm membrane and transferred to sampler vials to be analyzed on a UPLC-MS system (UPLC, ACQUITY UPLC H-Class PLUS Bio System, Waters Corporation, MA, USA; MS, Q-Exactive, Thermo Scientific, NJ, USA) equipped with a heated electrospray ionization (HESI) source. The UPLC separation was operated on a BEH C18 column (2.1 × 100 mm, 1.7 µm, Waters Corporation), with mobile phase comprised of 0.1% formic acid water solution (A) and 0.1% formic acid acetonitrile solution (B), flow rate set as 0.3 mL/min, column temperature set at 35 °C, and injection volume set as 5 µL. The MS analysis was performed in an electrospray ionization positive mode, and data were acquired with full scan using a mass resolution of 70,000 and a scan range of 50 to 750 m/z. For MS/MS analysis, an isolation window of 2.0 m/z and a mass resolution of 17,500 were selected.

Statistical Analysis

For the metabolomics data, the software SIEVE 2.1 (Thermo Scientific) and Progenesis Q1 (Nonlinear Dynamics, Waters Corporation) were used for raw data processing, including background subtraction, peak alignment, identification, and normalization with Pareto scaling to get the standardized relative abundance of the metabolites in different samples. Then principal components analysis (PCA) was conducted using SIMCA-P 13 software (Umetrics, Umea, Sweden), and one-way ANOVA was conducted using JMP Pro 14.3.0 (SAS Institute Inc., Cary, NC, USA) to compare the abundance of metabolites among different treatments. The metabolites with fold change > 1.5, CV < 20% and $P < 0.05$ were selected for further identification by comparison of the ion features in the experimental samples to the chemical standard entries in reference libraries (METLIN, HMDB and KEGG) that included retention time, molecular weight (m/z) as well as their associated MS/MS spectra. Boxplot was plotted using the ggplot2 package in R software (<http://cran.r-project.org/>, version 4.0.2) to illustrate the concentrations of the compounds that identified with significantly difference among the three treatments. Those compounds were then imported into the module of pathway analysis in Metaboanalyst 3.0

(<https://www.metaboanalyst.ca/MetaboAnalyst/upload/PathUploadView.xhtml>) to generate the pathway topology analysis. The metabolic pathway with impact value greater than 0.1 was characterized as the significantly relevant pathway [19].

For the other data, normality and homogeneity of variance were checked using the normal probability plot and residual plot from JMP Pro 14.3.0, and outliers were removed before further analysis. For N and energy balance data and serum hormone and biochemical markers data collected in Trial 1, a statistical model including ambient temperature as the only fixed effect and chamber as the random effect was fitted using the Fit Model function in JMP Pro 14.3.0. Tukey's HSD test was used to separate the least square means among treatments with significantly different effects. Moreover, linear and quadratic effects of increased ambient temperature on responses were tested by polynomial contrast using the Fit Y by X function in JMP Pro 14.3.0. To better illustrate the comprehensive effects of ambient temperature and pig BW, the N and energy balance data collected in Trial 2 were combined with some corresponding data (those under the same temperature settings) in Trial 1 for analysis. A statistical model included the main effects of ambient temperature (18 °C, 23 °C, 27 °C, and 32 °C) and BW (25 kg and 65 kg), and their interaction effect as fixed effects, and included the chamber as the random effect. The Fit Model function in JMP Pro 14.3.0 was used to conduct the two-way ANOVA, and Tukey's HSD test was used to separate the least square means among treatments with significantly different main effects only when the interaction effect was not significant. Moreover, equations were developed to predict the voluntary feed intake (VFI), ME_i , RE_p and RE_L using metabolic body weight ($BW^{0.6}$) and ambient temperature as predictors based on some modifications of the equations from Quiniou et al. [20]:

$$VFI \text{ or } ME_i = a + b \times BW^{0.6} + c \times (BW^{0.6})^2 + d \times T + e \times T^2 + f \times BW^{0.6} \times T,$$

$$RE_p \text{ or } RE_L = a + b \times BW^{0.6} + c \times (BW^{0.6})^2 + d \times T + e \times T^2 + f \times BW^{0.6} \times T + g \times ME_i.$$

All the data obtained in Trial 1 and 2 were combined and used for model development. Covariance analysis was used to further confirm the quadratic effects of BW and T on response variables, and coefficients were estimated by Gauss-Newton analysis using the Nonlinear Modelling function in JMP Pro 14.3.0. The predicted vs. actual plot and predicted vs. residuals plot were used to check the goodness of fit of the models. For all the analysis, $P < 0.05$ was considered as significantly different.

Results

Energy partition in pigs at 25 kg under different ambient temperatures

The energy partition of modern growing pigs at 25 kg kept at 18 °C, 21 °C, 23 °C, 27 °C, 29 °C and 32 °C were present in Table 2. As the ambient temperature increased from 18 °C to 32 °C, the DMI, N intake, fecal N output, N retention, ME_i, THP and RE_p of growing pigs at 25 kg all showed linearly and quadratically decreased pattern ($P < 0.05$). Specifically, pigs raised at 18 °C had greater DMI, N intake, N retention, ME_i, THP and RE_p compared with those raised at 32 °C, while pigs kept at 18 °C also had greater THP compared with those kept at 29 °C ($P < 0.05$). Moreover, pigs raised at 18 °C and 21 °C exhibited greater fecal N output than those raised at 27 °C, and pigs kept at 23 °C also exhibited greater RE_p than those kept at 32 °C ($P < 0.05$). On the other hand, as the ambient temperature increased from 18 °C to 32 °C, the OXCHO and RQ at fasting state linearly and quadratically increased, with pigs under 32 °C showed greater OXCHO and RQ during fasting compared with pigs under 18 °C, 21 °C and 23 °C ($P < 0.05$). Otherwise, no significant changes were observed on other parameters related to energy balance and utilization under different ambient temperatures ($P > 0.05$).

Table 2

Nitrogen and energy balance of barrows at 25 kg kept at different ambient temperatures ¹.

Items	Ambient temperature (°C)						SEM	Pvalue ⁷		
	18	21	23	27	29	32		ANOVA	Linear	Quadratic
Average body weight, kg	28.7	28.7	27.0	28.7	28.8	28.7	0.79	0.587	0.709	0.622
Dry matter intake, kg/d	1.32 ^a	1.24 ^{ab}	1.21 ^{ab}	1.05 ^{ab}	1.16 ^{ab}	0.99 ^b	0.06	0.030	< 0.001	0.002
Nitrogen balance, g/d										
Intake	41.9 ^a	39.3 ^{ab}	38.4 ^{ab}	33.4 ^{ab}	36.8 ^{ab}	31.6 ^b	2.05	0.030	< 0.001	0.002
Fecal output	6.7 ^a	6.6 ^a	5.9 ^{ab}	4.3 ^b	4.6 ^{ab}	4.8 ^{ab}	0.47	0.004	< 0.001	< 0.001
Urine output	5.4	4.6	3.2	4.9	6.0	3.8	0.92	0.327	0.867	0.926
Retention	29.8 ^a	28.0 ^{ab}	29.2 ^{ab}	24.7 ^{ab}	26.2 ^{ab}	21.7 ^b	1.73	0.040	< 0.001	0.003
Energy balance, kJ/kg BW ^{0.6} /d										
ME intake	2699 ^a	2510 ^{ab}	2555 ^{ab}	2197 ^{ab}	2426 ^{ab}	2035 ^b	133.3	0.025	< 0.001	0.004
Total heat production	1133 ^a	1093 ^{ab}	1075 ^{ab}	1000 ^{ab}	947 ^b	925 ^b	55.2	0.004	0.002	0.011
Oxidation of carbohydrates _{fed state} ²	1304	1330	1550	1236	1100	1192	124.2	0.084	0.168	0.329
Oxidation of fat _{fed state} ³	-304	-361	-587	-370	-274	-263	119.1	0.197	0.388	0.331
Total heat production _{adjusted} ⁴	1010	1063	1034	1123	954	1069	86.8	0.629	0.875	0.953
Fasting heat production (FHP)	716	713	628	713	666	698	48.6	0.809	0.758	0.748
Oxidation of carbohydrates _{fast state} ²	189 ^b	225 ^b	210 ^b	283 ^{ab}	267 ^{ab}	404 ^a	44.9	0.014	0.002	0.004
Oxidation of fat _{fast state} ³	472	465	392	400	339	300	62.0	0.233	0.017	0.058
Energy retention (RE)	1565	1417	1480	1202	1478	1145	143.9	0.193	0.059	0.172

^{a,b} Within a row means with different superscript letters differ at $P < 0.05$.¹ ANOVA, analysis of variance; DE, digestible energy; GE, gross energy; ME: metabolizable energy; NE: net energy; SEM, standard error of the mean.² Oxidation of carbohydrates (kJ/kg BW^{0.6}/day) = $[-2.968 \times \text{O}_2 \text{ (L)} + 4.147 \times \text{CO}_2 \text{ (L)} - 1.761 \times \text{CH}_4 \text{ (L)} - 2.446 \times \text{Urinary N (g)}] \times 17.58/\text{BW}^{0.6}$.³ Oxidation of fat (kJ/kg BW^{0.6}/day) = $[1.719 \times \text{O}_2 \text{ (L)} - 1.719 \times \text{CO}_2 \text{ (L)} - 1.719 \times \text{CH}_4 \text{ (L)} - 1.963 \times \text{Urinary N (g)}] \times 39.76/\text{BW}^{0.6}$.⁴ Total heat production adjusted = Total heat production was adjusted to the same ME intake at 2.4 MJ ME/kg BW^{0.6}/d.⁵ RE_p = Energy retention as protein (kJ/kg BW^{0.6}/d) = $[\text{N intake (g)} - \text{fecal N output (g)} - \text{urine N output (g)}] \times 6.25 \times 23.86 \text{ (kJ/g)}/\text{BW}^{0.6}$.⁶ RE_L = Energy retention as fat (kJ/kg BW^{0.6}/d) = $[\text{RE (kJ)} - \text{energy retention as protein (kJ)}]/\text{BW}^{0.6}$.⁷ Linear and Quadratic represents the polynomial contrasts analysis on linear and quadratic effects of the ambient temperatures.

Items	Ambient temperature (°C)						SEM	P-value ⁷		
	18	21	23	27	29	32		ANOVA	Linear	Quadratic
RE _p ⁵	594 ^a	558 ^{ab}	602 ^a	491 ^{ab}	521 ^{ab}	431 ^b	32.8	0.013	< 0.001	0.001
RE _L ⁶	971	860	879	715	957	714	121.0	0.409	0.220	0.471
Respiratory quotient										
Fed state	1.07	1.09	1.09	1.08	1.05	1.12	0.03	0.576	0.520	0.635
Fast state	0.80 ^b	0.80 ^b	0.80 ^b	0.83 ^{ab}	0.83 ^{ab}	0.87 ^a	0.02	0.025	0.003	0.007
Energy utilization, %										
DE/GE	84.9	84.3	84.9	86.7	87.6	84.9	1.13	0.293	0.211	0.307
Urine energy/DE	1.0	1.1	1.1	1.2	1.4	1.3	0.18	0.709	0.114	0.283
Methane energy/DE	0.35	0.42	0.38	0.50	0.40	0.43	0.07	0.829	0.426	0.559
ME/DE	98.6	98.5	98.6	98.3	98.2	98.5	0.21	0.647	0.274	0.411
NE/ME	85.1	85.4	83.4	84.1	88.7	86.0	2.30	0.577	0.441	0.709
RE/ME	57.9	55.7	56.9	53.2	60.2	55.5	3.62	0.629	0.875	0.953
RE _p /ME	22.0	22.2	23.6	22.2	21.5	22.1	0.68	0.347	0.522	0.513
RE _L /ME	35.9	33.5	33.3	31.1	38.8	30.4	3.46	0.434	0.554	0.839
FHP/ME	26.6	29.2	22.9	27.9	27.9	31.6	2.47	0.239	0.226	0.294
^{a,b} Within a row means with different superscript letters differ at $P < 0.05$.										
¹ ANOVA, analysis of variance; DE, digestible energy; GE, gross energy; ME: metabolizable energy; NE: net energy; SEM, standard error of the mean.										
² Oxidation of carbohydrates (kJ/kg BW ^{0.6} /day) = $[-2.968 \times \text{O}_2 \text{ (L)} + 4.147 \times \text{CO}_2 \text{ (L)} - 1.761 \times \text{CH}_4 \text{ (L)} - 2.446 \times \text{Urinary N (g)}] \times 17.58/\text{BW}^{0.6}$.										
³ Oxidation of fat (kJ/kg BW ^{0.6} /day) = $[1.719 \times \text{O}_2 \text{ (L)} - 1.719 \times \text{CO}_2 \text{ (L)} - 1.719 \times \text{CH}_4 \text{ (L)} - 1.963 \times \text{Urinary N (g)}] \times 39.76/\text{BW}^{0.6}$.										
⁴ Total heat production adjusted = Total heat production was adjusted to the same ME intake at 2.4 MJ ME/kg BW ^{0.6} /d.										
⁵ RE _p = Energy retention as protein (kJ/kg BW ^{0.6} /d) = $[\text{N intake (g)} - \text{fecal N output (g)} - \text{urine N output (g)}] \times 6.25 \times 23.86 \text{ (kJ/g)}/\text{BW}^{0.6}$.										
⁶ RE _L = Energy retention as fat (kJ/kg BW ^{0.6} /d) = $[\text{RE (kJ)} - \text{energy retention as protein (kJ)}]/\text{BW}^{0.6}$.										
⁷ Linear and Quadratic represents the polynomial contrasts analysis on linear and quadratic effects of the ambient temperatures.										

Energy partition in pigs at different BW under different ambient temperatures

Comparison on energy partition of modern growing pigs at 25 kg and 65 kg stayed at 18 °C, 23 °C, 27 °C, and 32 °C were present in Table 3. There were significant interaction effects between BW and ambient temperature on DMI, N balance (including N intake, fecal N output, urine N output and N retention), ME_i, THP adjusted to the same ME_i, RE, RE_p, RE_L, RQ in fed state, NE/ME, RE/ME and RE_L/ME ($P < 0.05$). However, the ME_i, THP adjusted to the same ME_i, RE, RE_p, RE_L, RE/ME and RE_L/ME did not exhibit difference between pigs at 25 kg and those at 65 kg, and the NE/ME did not show difference among pigs under 18 °C, 23 °C, 27 °C, and 32 °C ($P > 0.05$).

Table 3

Comparison between barrows at 25 kg and 65 kg on nitrogen and energy balance kept at different ambient temperatures ¹.

Items	Ambient temperature (°C)				Body weight (kg)		SEM	P-value ⁷		
	18	23	27	32	25	65		BW	T	BW × T
Dry matter intake, kg/d	1.81	1.75	1.39	1.11	1.14	1.88	0.08	< 0.001	< 0.001	< 0.001
Nitrogen balance, g/d										
Intake	57.3	55.4	44.0	35.2	36.4	59.6	2.44	< 0.001	< 0.001	< 0.001
Fecal output	8.9	7.4	5.6	4.9	5.4	8.0	0.57	< 0.001	< 0.001	0.011
Urine output	13.3	7.7	8.6	5.8	4.6	13.0	1.72	< 0.001	0.001	0.003
Retention	34.6	38.0	29.8	24.5	26.3	37.2	1.92	< 0.001	< 0.001	0.042
Energy balance, kJ/kg BW ^{0.6} /d										
ME intake	2737	2924	2193	1751	2375	2427	119.7	0.514	< 0.001	< 0.001
Total heat production	1168 ^a	1120 ^a	998 ^b	894 ^b	1024	1066	53.8	0.130	< 0.001	0.601
Oxidation of carbohydrates _{fed state} ²	1251 ^{ab}	1483 ^a	1162 ^b	1013 ^b	1320	1134	114.2	0.022	0.002	0.424
Oxidation of fat _{fed state} ³	-274	-399	-305	-140	-310	-249	97.8	0.397	0.104	0.438
Total heat production _{adjusted} ⁴	1032	941	1111	1238	1057	1104	76.9	0.333	0.001	0.007
Fasting heat production (FHP)	725	723	678	712	709	710	60.6	0.991	0.860	0.875
Oxidation of carbohydrates _{fast state} ²	150	210	201	258	252	157	44.2	0.001	0.056	0.149
Oxidation of fat _{fast state} ³	375	349	334	300	385	294	65.1	0.060	0.732	0.424

^{a-c} Within a row means with different superscript letters differ at $P < 0.05$. Different means are separated with superscripts in the table to illustrate the significant main effects only when the interaction effect is not significant.

¹ DE, digestible energy; GE, gross energy; ME: metabolizable energy; NE: net energy; SEM, standard error of the mean.

² Oxidation of carbohydrates (kJ/kg BW^{0.6}/day) = $[-2.968 \times \text{O}_2 \text{ (L)} + 4.147 \times \text{CO}_2 \text{ (L)} - 1.761 \times \text{CH}_4 \text{ (L)} - 2.446 \times \text{Urinary N (g)}] \times 17.58/\text{BW}^{0.6}$.

³ Oxidation of fat (kJ/kg BW^{0.6}/day) = $[1.719 \times \text{O}_2 \text{ (L)} - 1.719 \times \text{CO}_2 \text{ (L)} - 1.719 \times \text{CH}_4 \text{ (L)} - 1.963 \times \text{Urinary N (g)}] \times 39.76/\text{BW}^{0.6}$.

⁴ Total heat production adjusted = Total heat production was adjusted to the same ME intake at 2.4 MJ ME/kg BW^{0.6}/d.

⁵ RE_p = Energy retention as protein (kJ/kg BW^{0.6}/d) = $[\text{N intake (g)} - \text{fecal N output (g)} - \text{urine N output (g)}] \times 6.25 \times 23.86 \text{ (kJ/g)}/\text{BW}^{0.6}$.

⁶ RE_L = Energy retention as fat (kJ/kg BW^{0.6}/d) = $[\text{RE (kJ)} - \text{energy retention as protein (kJ)}]/\text{BW}^{0.6}$.

⁷ BW, T, and BW × T represents the main effects of body weight, the main effect of ambient temperature, and the interaction effect between body weight and ambient temperature, respectively.

Items	Ambient temperature (°C)				Body weight (kg)		SEM	<i>P</i> -value ⁷		
	18	23	27	32	25	65		BW	T	BW × T
Energy retention (RE)	1569	1804	1195	857	1351	1361	124.0	0.896	< 0.001	< 0.001
RE _p ⁵	581	663	469	364	528	511	37.1	0.517	< 0.001	0.015
RE _L ⁶	988	1141	724	540	822	874	101.9	0.437	< 0.001	< 0.001
Respiratory quotient										
Fed state	1.08	1.09	1.07	1.05	1.08	1.06	0.02	0.059	0.140	0.013
Fast state	0.80	0.80	0.81	0.84	0.82	0.81	0.02	0.496	0.084	0.893
Energy utilization, %										
DE/GE	85.7	87.1	87.8	86.5	85.7	87.8	0.91	0.003	0.171	0.230
Urine energy/DE	1.3	1.1	1.4	1.6	1.1	1.6	0.24	0.006	0.245	0.158
Methane energy/DE	0.41 ^b	0.42 ^{ab}	0.63 ^{ab}	0.64 ^a	0.42	0.63	0.08	0.002	0.011	0.188
ME/DE	98.3	98.4	97.9	97.8	98.4	97.8	0.30	0.006	0.228	0.112
NE/ME	78.5	81.6	78.5	76.3	84.8	72.7	2.00	< 0.001	0.084	0.006
RE/ME	57.0	60.8	53.7	48.4	56.0	54.0	3.20	0.333	0.001	0.007
RE _p /ME	21.2	22.8	22.2	21.3	22.3	21.5	0.81	0.154	0.139	0.534
RE _L /ME	35.8	38.0	32.2	29.3	33.7	34.0	3.32	0.881	0.037	0.031
FHP/ME	26.8 ^{bc}	22.6 ^c	30.4 ^b	39.4 ^a	27.8	31.8	3.17	0.049	< 0.001	0.259
^{a-c} Within a row means with different superscript letters differ at <i>P</i> < 0.05. Different means are separated with superscripts in the table to illustrate the significant main effects only when the interaction effect is not significant.										
¹ DE, digestible energy; GE, gross energy; ME: metabolizable energy; NE: net energy; SEM, standard error of the mean.										
² Oxidation of carbohydrates (kJ/kg BW ^{0.6} /day) = [-2.968 × O ₂ (L) + 4.147 × CO ₂ (L) - 1.761 × CH ₄ (L) - 2.446 × Urinary N (g)] × 17.58/BW ^{0.6} .										
³ Oxidation of fat (kJ/kg BW ^{0.6} /day) = [1.719 × O ₂ (L) - 1.719 × CO ₂ (L) - 1.719 × CH ₄ (L) - 1.963 × Urinary N (g)] × 39.76/BW ^{0.6} .										
⁴ Total heat production adjusted = Total heat production was adjusted to the same ME intake at 2.4 MJ ME/kg BW ^{0.6} /d.										
⁵ RE _p = Energy retention as protein (kJ/kg BW ^{0.6} /d) = [N intake (g) - fecal N output (g) - urine N output (g)] × 6.25 × 23.86 (kJ/g)/BW ^{0.6} .										
⁶ RE _L = Energy retention as fat (kJ/kg BW ^{0.6} /d) = [RE (kJ) - energy retention as protein (kJ)]/BW ^{0.6} .										
⁷ BW, T, and BW × T represents the main effects of body weight, the main effect of ambient temperature, and the interaction effect between body weight and ambient temperature, respectively.										

For the other parameters without interaction effects, pigs at 65 kg showed greater DE/GE, urine energy/DE, methane energy/DE and FHP/ME, but lower OXCHO at both fed state and fast state, and ME/DE compared with pigs at 25 kg (*P* < 0.05). In addition, pigs stayed at 27 °C and 32 °C demonstrated lower THP and lower OXCHO at fed state than pigs stayed at 18 °C and 23 °C, and pigs stayed at 23 °C, respectively; while pigs stayed at 32 °C exhibited greater methane energy/DE and greater FHP/ME than pigs stayed at 18 °C, and pigs stayed under the other three ambient temperatures (*P* < 0.05).

Modelling the voluntary feed intake and energy partition considering pigs' BW and ambient temperature

The quadratic effects of BW and T as well as their interaction effect on response variables were further confirmed. Models developed in the current study to predict VFI, ME_i, RE_p and RE_L using both BW and ambient temperature (T) as predictors were:

$$\text{VFI (kg/d)} = -7.72 + 1.54 \times \text{BW}^{0.6} - 0.059 \times (\text{BW}^{0.6})^2 + 0.15 \times T - 0.0022 \times T^2 - 0.0088 \times \text{BW}^{0.6} \times T \quad (R^2 = 0.86);$$

$$\text{ME}_i \text{ (kJ/kg BW}^{0.6}\text{/d)} = -9242.45 + 2042.05 \times \text{BW}^{0.6} - 91.95 \times (\text{BW}^{0.6})^2 + 242.32 \times T - 4.56 \times T^2 - 8.33 \times \text{BW}^{0.6} \times T \quad (R^2 = 0.67);$$

$$\text{RE}_p \text{ (kJ/kg BW}^{0.6}\text{/d)} = -233.38 - 67.28 \times \text{BW}^{0.6} + 3.16 \times (\text{BW}^{0.6})^2 + 39.58 \times T - 0.76 \times T^2 - 0.26 \times \text{BW}^{0.6} \times T + 0.26 \times \text{ME}_i \quad (R^2 = 0.86);$$

$$\text{RE}_L \text{ (kJ/kg BW}^{0.6}\text{/d)} = -465.10 + 8.10 \times \text{BW}^{0.6} - 1.03 \times (\text{BW}^{0.6})^2 - 53.73 \times T + 1.34 \times T^2 + 0.67 \times \text{BW}^{0.6} \times T + 0.69 \times \text{ME}_i \quad (R^2 = 0.85);$$

which were present in Table 4. The estimated VFI, ME_i, RE_p and RE_L of pigs at 25 kg and 65 kg under ambient temperatures from 18 °C to 32 °C based on these models were plotted in Fig. 1A-D, which illustrated that feed intake and energy retention in pigs at 65 kg were more easily impaired by high temperatures than in pigs at 25 kg. Meanwhile, the models for VFI and ME_i were compared with the below models developed previously (Fig. 1A and 1B):

Table 4

□ Prediction equations for voluntary feed intake, metabolizable energy intake, energy retention as protein and energy retention as lipid using body weight and ambient temperature as predictors.

No.	Models	Coefficients estimation ²							RMSE	R ²	P-value
		a	b	c	d	e	f	g			
1	VFI = a + b × BW ^{0.6} + c × (BW ^{0.6}) ² + d × T + e × T ² + f × BW ^{0.6} × T	-7.72	1.54	-0.059	0.15	-0.0022	-0.0088	/	0.18	0.86	< 0.001
2	ME _i = a + b × BW ^{0.6} + c × (BW ^{0.6}) ² + d × T + e × T ² + f × BW ^{0.6} × T	-9242.45	2042.05	-91.95	242.32	-4.56	-8.33	/	316.30	0.67	< 0.001
3	RE _p = a + b × BW ^{0.6} + c × (BW ^{0.6}) ² + d × T + e × T ² + f × BW ^{0.6} × T + g × ME _i	-233.38	-67.28	3.16	39.58	-0.76	-0.26	0.26	61.66	0.86	< 0.001
4	RE _L = a + b × BW ^{0.6} + c × (BW ^{0.6}) ² + d × T + e × T ² + f × BW ^{0.6} × T + g × ME _i	-465.10	8.10	-1.03	-53.73	1.34	0.67	0.69	134.58	0.85	< 0.001
¹ BW, body weight (kg); ME _i , metabolizable energy intake (kJ/kg BW ^{0.6} /d); RE _L , energy retention as lipid (kJ/kg BW ^{0.6} /d); RE _p , energy retention as protein (kJ/kg BW ^{0.6} /d); RMSE, root mean square error; T, ambient temperature (°C); VFI: voluntary feed intake (kg).											
² Coefficients are estimated using Gauss-Newton analysis.											

$$\text{VFI (kg/d)} = -1.264 + 0.0736 \times \text{BW} - 0.00026 \times \text{BW}^2 + 0.117 \times T - 0.0024 \times T^2 - 0.00095 \times \text{BW} \times T \quad (\text{from Quiniou et al. [20]});$$

$$\text{ME}_i \text{ (kJ/d)} = -26811.2 + 1268.4 \times \text{BW} - 5.215 \times \text{BW}^2 + 1572.3 \times T - 27.48 \times T^2 - 15.7 \times \text{BW} \times T \quad (\text{from Guo [21]});$$

Nearly parallel VFI curves were present between models in the current study and those built previously, but narrower gaps between ME_i curves representing different BWs were found in our study compared with those from previous study especially under high ambient temperatures. Moreover, the goodness-of-fit of those developed models were shown in Fig. 2 using predicted vs. actual plot and predicted vs. residuals plot, indicating no problems on fitness of the models developed in the current study.

Changes in hormone and biochemical markers levels in serum of pigs at 25 kg under different ambient temperatures

The concentrations of hormone and biomedical markers in serum of modern growing pigs at 25 kg kept at 18 °C, 21 °C, 23 °C, 27 °C, 29 °C and 32 °C were present in Table 5. The levels of cortisol, T₃, T₄, HDL and TC in pig serum showed patterns of linearly and quadratically decreased as the ambient temperature increased from 18 °C to 32 °C, with the T₄ level at 18 °C greater than that at 27 °C, 29 °C and 32 °C, the HDL level at 18 °C greater than that at 27 °C and 29 °C, and the TC level at 18 °C, 21 °C and 23 °C greater than that at 27 °C ($P < 0.05$). Moreover, the glucagon and AST levels in pig serum quadratically changed as the ambient temperature increased from 18 °C to 32 °C, with the AST level at 29 °C greater than that at 18 °C ($P < 0.05$), and the glucagon level at 27 °C tended to be the lowest among all the treatment groups ($P = 0.054$).

Table 5
Hormone and biochemical markers levels in serum of barrows at 25 kg kept at different ambient temperature ¹.

Items	Ambient temperature (°C)						SEM	P-value ²		
	18	21	23	27	29	32		ANOVA	Linear	Quadratic
Cortisol, ng/ml	61.8	67.3	61.6	59.2	47.3	36.9	7.87	0.058	0.006	0.010
Glucagon, pg/ml	89.6	83.9	76.6	63.6	83.6	98.1	6.73	0.054	0.673	0.012
Growth hormone, ng/ml	4.53	5.23	6.56	4.06	4.97	5.97	0.98	0.571	0.707	0.930
Insulin, µIU/ml	8.69	8.00	8.68	7.59	7.22	8.09	0.54	0.470	0.171	0.335
Thyroxine, ng/ml	58.6 ^a	46.2 ^{ab}	45.7 ^{ab}	41.3 ^{bc}	33.6 ^{bc}	31.0 ^c	3.66	<0.001	<0.001	<0.001
Triiodothyronine, ng/ml	0.63	0.62	0.59	0.55	0.59	0.52	0.03	0.061	0.010	0.039
Albumin, µmol/L	35.7	35.8	35.4	33.8	35.0	37.2	0.98	0.303	0.661	0.212
ALT, U/L	61.2	53.8	60.4	50.5	55.5	56.0	3.34	0.337	0.244	0.309
AST, U/L	35.2 ^b	36.8 ^{ab}	48.5 ^{ab}	54.3 ^{ab}	55.5 ^a	39.2 ^{ab}	4.62	0.013	0.082	0.005
Globulin, µmol/L	34.1	31.6	37.2	35.1	34.9	33.0	1.27	0.128	0.937	0.438
HDL, mmol/L	0.55 ^a	0.49 ^{abc}	0.53 ^{ab}	0.40 ^c	0.43 ^{bc}	0.46 ^{abc}	0.03	0.004	0.004	0.006
LDL, mmol/L	1.55	1.36	1.60	1.34	1.45	1.32	0.07	0.052	0.064	0.168
Total cholesterol, mmol/L	2.47 ^a	2.30 ^a	2.40 ^a	2.05 ^b	2.25 ^{ab}	2.26 ^{ab}	0.05	0.002	0.017	0.007
Triglyceride, mmol/L	0.54	0.44	0.48	0.54	0.49	0.40	0.04	0.134	0.198	0.351
Total protein, g/L	69.8	67.4	68.8	71.5	69.1	70.2	2.06	0.845	0.556	0.834
Urea, mmol/L	4.21	3.84	4.50	3.76	3.99	4.23	0.18	0.306	0.863	0.380
^{a-c} Within a row means with different superscript letters differ at $P < 0.05$.										
¹ ALT, glutamic-pyruvic transaminase; AST, glutamic oxalacetic transaminase; ANOVA, analysis of variance; HDL, high density lipoprotein; LDL, low density lipoprotein; SEM, standard error of the mean.										
² Linear and Quadratic represents the polynomial contrasts analysis on linear and quadratic effects of the ambient temperatures.										

Metabolomics profiles in plasma of pigs at 25 kg under different ambient temperatures

The PCA score plot showed that the plasma samples from pigs stayed at low (18 °C), neutral (23 °C) and high (32 °C) ambient temperatures obviously clustered within the 95% confidence interval (Fig. 3). The PC1 and PC2 accounted for 41.9% and 9.98% of the total

variance of the data, respectively, indicating there were major difference among the metabolomics profiles of the three treatment groups.

A total of 13 compounds were identified with different intensities among the three treatment groups by comparing to the standard entries in reference libraries (Table 6), which include categories of amino acid, fatty acid and steroid. To better illustrate the changes of those compounds in the treatment groups, data were visualized through boxplot in Fig. 4. Compared to pigs stayed at the neutral temperature (23 °C) or low temperature (18 °C), the concentrations of (2'E,4'Z,7'Z,8E)-colnelenic acid, 3-beta-hydroxy-5-cholestenoate, adrenic acid, dihydrocortisol and indoleacetic acid all dramatically up-regulated, while the concentrations of 20-hydroxyeicosatetraenoic acid, beta-sitosterol, cortisol, deoxyuridine, leukotriene C4, phenylacetyl glycine and spermidine all greatly down-regulated in plasma of pigs stayed at the high temperature (32 °C). In addition, compared to pigs stayed at neutral temperature, the levels of beta-sitosterol markedly up-regulated, while the levels of (2'E,4'Z,7'Z,8E)-colnelenic acid, cortisol, L-histidine, phenylacetyl glycine and spermidine slightly down-regulated in plasma of pigs stayed at the low temperature (18 °C).

Table 6

Metabolites with significantly different concentrations in plasma of barrows at 25 kg kept at different ambient temperatures.

No.	Compounds	m/z	Formula	Fold change ¹			Pathway
				High/Neutral	Low/Neutral	High/Low	
1	(2'E,4'Z,7'Z,8E)-Colnelenic acid	275.1994	C ₁₈ H ₂₈ O ₃	4.97	0.84	5.91	alpha-Linolenic acid metabolism
2	20-Hydroxyeicosatetraenoic acid	303.2305	C ₂₀ H ₃₂ O ₃	0.48	1.21	0.40	Arachidonic acid metabolism
3	3-beta-Hydroxy-5-cholestenoate	399.3242	C ₂₇ H ₄₄ O ₃	2.39	1.22	1.96	Primary bile acid biosynthesis
4	Adrenic acid	355.2614	C ₂₂ H ₃₆ O ₂	4.83	1.11	4.35	Biosynthesis of unsaturated fatty acids
5	Beta-Sitosterol	397.3812	C ₂₉ H ₅₀ O	0.24	2.36	0.10	Steroid biosynthesis
6	Cortisol	363.2152	C ₂₁ H ₃₀ O ₅	0.35	0.82	0.43	Steroid hormone biosynthesis
7	Deoxyuridine	251.0629	C ₉ H ₁₂ N ₂ O ₅	0.81	1.24	0.66	Pyrimidine metabolism
8	Dihydrocortisol	382.2571	C ₂₁ H ₃₂ O ₅	2.62	1.30	2.02	Steroid hormone biosynthesis
9	Indoleacetic acid	176.0700	C ₁₀ H ₉ NO ₂	2.61	1.01	2.59	Tryptophan metabolism
10	L-Histidine	156.0763	C ₆ H ₉ N ₃ O ₂	1.04	0.63	1.66	Histidine metabolism/beta-Alanine metabolism/Aminoacyl-tRNA biosynthesis
11	Leukotriene C4	313.6596	C ₃₀ H ₄₇ N ₃ O ₉ S	0.56	1.14	0.49	Arachidonic acid metabolism
12	Phenylacetyl glycine	211.1070	C ₁₀ H ₁₆ N ₂ O ₄	0.57	0.79	0.73	Phenylalanine metabolism
13	Spermidine	146.1647	C ₇ H ₁₉ N ₃	0.32	0.75	0.43	beta-Alanine metabolism/Arginine and proline metabolism/Glutathione metabolism
¹ Fold change represents ratios of the normalized intensity of the metabolites in plasma of growing pigs at two different ambient temperatures. Low, ambient temperature at 18 °C; Neutral, ambient temperature at 23 °C; High, ambient temperature at 32 °C. Fold change > 1 represents the metabolite was up-regulated, and fold change < 1 represents the metabolite was down-regulated.							

According to the pathway analysis, the identified metabolites are involved in pathways related to amino acid and lipid metabolism including histidine metabolism, beta-alanine metabolism, arachidonic acid metabolism, steroid hormone biosynthesis, and so on. However, only the pathway of histidine metabolism had an impact value greater than 0.1 (impact value = 0.22), which is the cut-off value for relevance gained from the topology analysis.

Discussion

Effects of ambient temperature on voluntary feed intake and energy partition of modern growing pigs

Most of the studies focusing on the VFI and energy partition patterns under different ambient temperatures in growing pigs were carried out in the second half of the 20th century. With the rapid developments in pig breeding, research updates on such topics concerning modern growing pigs have been absent in recent years.

Pig belongs to the warm-blooded animal. Under low ambient temperatures, the increased feed intake and heat production can facilitate the maintenance of a constant body temperature [22]. The criteria of "low" temperature is defined according to the thermoneutral zone and the LCT value, which is usually estimated by BW. NRC [14] used the following equations to estimate the LCT in growing pigs: $LCT (^{\circ}C) = 17.9 - 0.0375 \times BW$. Based on the temperature setting in the current study, all the pigs used in the animal trial were kept above the LCT. We observed an averaged 30 g/d/ $^{\circ}C$ increase in DMI and 0.38 MJ/d/ $^{\circ}C$ increase in ME_i when the ambient temperature decreased from 27 $^{\circ}C$ to 18 $^{\circ}C$ for pigs with mean BW of 28 kg in the current study. Our results are similar to the VFI and energy intake patterns gained from Le Dividich et al. [23], who illustrated that the extra feed intake requirements were 32–33 g/d/ $^{\circ}C$ and the additional DE required to compensate for 1 $^{\circ}C$ drop in temperature between 28 $^{\circ}C$ and 20 $^{\circ}C$ and between 20 $^{\circ}C$ and 12 $^{\circ}C$ were 0.20 and 0.44 MJ/d in 30 kg growing pigs individually housed, respectively, but are different from the results from other studies. For example, Lefaucheur et al. [24] reported that an extra feed intake of 25 g/d/ $^{\circ}C$ was required to achieve similar rates of weight gain for individual pig at 30 to 90 kg when environmental temperature decreased from 28 $^{\circ}C$ to 12 $^{\circ}C$. The extra feed intake requirements estimated by Quiniou et al. [1] were 19 g/d/ $^{\circ}C$ between 12 $^{\circ}C$ and 24 $^{\circ}C$ for 30–90 kg pigs grouped-housed. Those different results may be attributed to the discrepancies in modern pig breeds and management conditions in pig farm nowadays, leading to more sensitive to the low environmental temperatures.

As the ambient temperature keeps raising, the heat production of the pig body becomes a "burden", and pigs can keep the body temperature fluctuate in a small range by increasing the evaporation heat loss and decreasing the heat production [22]. However, due to the degradation of the sweat gland, the capacity of heat dissipation through evaporation by pigs is limited [22]. Therefore, under high ambient temperatures, the feed intake of pigs will decrease dramatically to control their heat production [22]. For example, Collin et al. [25] showed that the VFI remained almost constant between 19 $^{\circ}C$ and 25 $^{\circ}C$, and proportionally decreased 33 g/day/ $^{\circ}C$ between 25 $^{\circ}C$ and 35 $^{\circ}C$ for pigs at 20 kg. In the current study, we observed an averaged 56 g/d/ $^{\circ}C$ in DMI and 0.90 MJ/d/ $^{\circ}C$ decrease in ME_i when the ambient temperature increased from 29 $^{\circ}C$ to 32 $^{\circ}C$ for pigs with mean BW of 28 kg, but also observed an averaged 55 g/d/ $^{\circ}C$ in DMI and 0.80 MJ/d/ $^{\circ}C$ increase in ME_i when the ambient temperature increased from 27 $^{\circ}C$ to 29 $^{\circ}C$. The same pattern was also observed in THP adjusted into the same ME_i level. Some previous studies also reported increased THP under high environmental temperatures, which could be attributed to the extra energy expenditure used for additional activity such as more frequent breathing and heart rating to facilitate the heat dissipation [9]. For instance, Close and Mount [26] illustrated that the THP was the lowest during 20 $^{\circ}C$ to 25 $^{\circ}C$, and gradually increased when the temperature reached 30 $^{\circ}C$. The above conflict in heat production results may rely on the specific temperature range, BW and feeding level of the pigs [21]. Moreover, the increased consumption of dietary energy by pigs for heat dissipation in high temperature environment also leads to the decreased energy utilization efficiency.

The environmental temperature could affect the partition of ME_i , specifically, into maintenance energy, energy for thermoregulation, and energy for growth (the RE) in growing pigs. In the current study, no significant influence of ambient temperatures on maintenance energy (ME_m , estimated by FHP), RE and the partial energy utilization efficiency, including partial efficiency for digestion (DE/GE), metabolism (ME/DE), net utilization (NE/ME), retention (RE/ME) and maintenance (FHP/ME), were observed. However, the ambient temperature altered the OXCHO and RQ during fast state as well as the RE_p , with OXCHO increased from ~ 200 to ~ 400 kJ/kg $BW^{0.75}/d$, RE_p decreased from ~ 600 to ~ 430 kJ/kg $BW^{0.75}/d$, and RQ increased from 0.80 to 0.87, when environment temperature increased from 18 $^{\circ}C$ to 32 $^{\circ}C$, reflecting the enhanced maintenance energy partition at low ambient temperature and reduced protein synthesis at high ambient temperature. According to the previous study from Close [27], when the environmental temperature increased from 10 $^{\circ}C$ to 30 $^{\circ}C$, the ME_m decreased from 723 to 469 kJ/kg $BW^{0.75}/d$, and the RE efficiency (RE/ME) decreased from 0.79 to 0.63, with a ME_m of 440 kJ/kg $BW^{0.75}/d$ and a RE efficiency of 0.67 at the thermoneutral temperature of 25 $^{\circ}C$. In addition, both the partial energy efficiency estimated for protein deposition and lipid deposition were similar at each environmental temperature and decreased from 0.78 at 10 $^{\circ}C$ to 0.63 at 30 $^{\circ}C$ [27]. The above results are somehow in according with our observations, indicating that under low ambient temperature, more energy intake were used to maintain the body temperature of pigs, while part of energy was used to dissipate the heat produced during protein and lipid synthesis under high ambient temperature, leading to the decreased partial energy efficiency. Due to the absence of dietary

energy gradients settings, the changes of partial efficiency for protein and lipid synthesis under different temperatures were not tested in the current study.

Effects of ambient temperature on nitrogen balance of modern growing pigs

In the current study, we also focused on the effects of ambient temperatures on N balance. For pigs raised from 18 °C to 32 °C, the changing patterns of N intake and fecal N output were the same as that of the adjusted THP, which has been discussed previously. Moreover, the ratio of fecal N output to total N intake ranged from 12.5–16.7%, and decreased from 18 °C to 29 °C, then increased from 29 °C to 32 °C. This range exactly matched the range of 16–12% for fecal N output to total N intake ratio reported by Guo [21] when temperature changed from 23 °C to 35 °C. Environmental temperature had no influence on urine N output in our study, which was also supported by Guo [21] and Verstegen et al. [3]. The ratio of N retention to total N intake ranged from 68.7% (32 °C) to 76.0% (23 °C), and is quite different from those of Verstegen et al. [3], who reported a range of 50%-54% of N retention to dietary N intake ratio, and was independent with ambient temperatures. Those discrepancies in results of N balance could be mainly ascribed to the differences in pig breeds and experimental conditions.

Modelling the energy partition of modern growing pigs with different BW under different ambient temperatures

It has been widely proved that pigs at different growth stages have distinct patterns of energy partition as the ambient temperatures change, since the critical temperatures differ for pigs with different BW [14]. In the current study, we found significant interaction effects between BW and temperature on DMI, N balance, ME_i , adjusted THP, RE and the partial efficiency for lipid deposition (RE_L/ME), with pigs at 65 kg showed greater the above indexes except for RE_p compared with pigs at 25 kg. Therefore, the quadratic models including the main effects of metabolic BW, ambient temperature and their interaction effects were used to fit the raw data in the current study and to predict the VFI, ME_i as well as RE_p and RE_L . Similar simulation equations have already been used to model the VFI [20], heat production and energy retention [1], and ME_i [21] in pigs exposed to low or high ambient temperatures. Based on those curvilinear models developed in our study, it can be clearly see that pigs at heavier BW were more sensitive to high temperatures on VFI, ME_i and protein and lipid deposition, which were partially supported by Renaudeau et al. [28], who analyzed the effects of high temperature on feed intake of pigs with different BW using meta-analysis based on 86 trials and 202 temperature treatments published from 1970 to 2009, and found that high temperature had a curvilinear effect on average daily feed intake, which was more pronounced in heavier pigs. In addition, the gaps between ME_i curves representing different BWs in our study were narrower compared with those from previous studies using pigs with older genotypes, suggesting that modern genotypes could be more sensitive to heat stress than older genotypes of lesser growth potential. Similarly, Renaudeau et al. [28] also reported that the effect of increased temperature was greater in more contemporary publications. However, one flaw of the models developed in the current study is that the BW data collected for simulation only came from 2 growth stages, and the narrow range of BW may produce bias when predicting the responses on pigs with other BWs.

Effects of ambient temperature on hormone and biochemical markers in serum of modern growing pigs

As the ambient temperature increased from 18 °C to 32 °C, there was linearly decreased cortisol, T_3 , T_4 , HDL and TC levels, linearly increased AST level, and quadratically changed glucagon level in serum of 25 kg pigs in the current study. Similar changes in physiological and biomedical parameters in plasma of growing pigs under heat stress were also reported previously [29, 30]. The decreased cortisol content in plasma under low temperature may indicate improved protein degradation to provide amino acids for gluconeogenesis [30]. Moreover, the decreased cortisol level and increased glucagon and AST levels in plasma may also act as indicators for the stress reaction of pigs under extreme environmental conditions [30]. In addition, the depressed thyroid hormone (T_3 and T_4) levels have been associated with lowered metabolic heat production in pigs kept at high environmental temperature [29]. Meanwhile, a reduction in HDL and TC, indicating the decreased β -oxidation of fat for energy purpose and favored hepatic synthesis of triglycerides, could also support the depressed plasma thyroid hormone levels in pigs reared at high ambient temperature [29].

Effects of ambient temperature on plasma metabolomics profiles of modern growing pigs

To simplify the analysis, only plasma samples from 18 °C, 23 °C and 32 °C were used for metabolomics analysis in the current study. Among the 13 compounds identified, more compounds showed significant changes in pigs at high ambient temperature than those at low ambient temperature when compared to the thermoneutral condition, indicating that modern growing pigs are more sensitive to heat

stress than cold stress reflected by the plasma metabolites. Another reason may be that the “low” temperature settings in the current study may not produce cold stress since it is above the LCT defined by NRC [14].

Under high ambient temperature, most of the metabolites with up-regulated expressions belong to fatty acids ((2'E,4'Z,7'Z,8E)-colnelenic acid, 3-beta-hydroxy-5-cholestenoate and adrenic acid), or involve in pathways related to lipid metabolism or fatty acid metabolism (dihydrocortisol), indicating elevated lipogenic pathways and suppressed fatty acid oxidation, which were also reflected by the levels of hormone and biochemical markers in serum in the current study, and was supported by the reports of Qu and Ajuwon [31], who showed greater serum linoleic and total polyunsaturated fatty acids levels in pigs stayed at 35 °C. On the other hand, the metabolites with down-regulated expressions at 32 °C are involved in amino acid metabolism (phenylacetylglycine and spermidine), or lipid metabolism (20-hydroxyeicosatetraenoic acid, beta-sitosterol and leukotriene C4), which may indicate the enhanced protein degradation and gluconeogenesis, as well as the depressed lipolysis. Interestingly, cortisol was also identified in the metabolomics analysis, with down-regulated level at high temperature, supporting the results in the plasma hormone assay. Furthermore, most of the metabolites in plasma only exhibited slightly changes in pigs at 18 °C compared to that at 23 °C, suggesting less stress under this temperature setting.

Conclusion

The current study provided updated mathematical models to predict the voluntary feed intake and metabolizable energy intake as well as new models to estimate the energy requirements for protein and lipid deposition of growing pigs (25 to 65 kg) under ambient temperatures of 18 to 32 °C. It also suggested that pigs at heavier body weight were more sensitive to high temperatures on energy intake and partition, and pigs with modern genotypes could be more sensitive to heat stress than pigs with older genotypes. The integrative analysis of indirect calorimetry and metabolomics profiling revealed that the decreased energy intake and total heat production under 32 °C are associated with suppressed fatty acid oxidation and elevated lipogenesis and protein degradation, reflected by biomarkers including up-regulated adrenic acid and down-regulated cortisol levels in plasma. Our findings provide practical tools to precisely formulate diets for modern growing pigs under different ambient temperatures, and can help to improve our knowledge on potential mechanisms of thermoregulation in modern pig breeds.

Abbreviations

ALT, glutamic-pyruvic transaminase; AST, glutamic oxalacetic transaminase; BW, body weight; CP, crude protein; DE, digestible energy; DM, dry matter; DMi, dry matter intake; EE, ether extract; FHP, fasting heat production; GE, gross energy; GH, growth hormone; HDL, high density lipoprotein; HP, heat production; LCT, lower critical temperature; LDL, low density lipoprotein; ME, metabolizable energy; ME_i, metabolizable energy intake; ME_m, maintenance energy; N, nitrogen; NE, net energy; OXCHO, oxidation of carbohydrates; OXF, oxidation of fat; PCA, principal components analysis; RE, energy retention; RE_L, energy retention as lipid; RE_p, energy retention as protein; RQ, respiratory quotient; T₃, triiodothyronine; T₄, thyroxine; TC, total cholesterol; TG, triglyceride; THP, total heat production; UCT, upper critical temperature; VFI, voluntary feed intake.

Declarations

Availability of data and materials

All the data were presented in the main manuscript and available to readers.

Ethics approval

All procedures used in this study were performed according to the guidelines for the ethical treatment of animal by the Institutional Animal Care and Use Committee of China Agricultural University (Beijing, China).

Competing interests

The authors declare no competing interests.

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Author's contributions

SZ performed the data analysis and drafted the manuscript. HG carried out the experiment and produced all the data. XKY participated in the experiments. JJW critically evaluated the manuscript. JJZ designed the experiment and critically evaluated the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Modelling feed intake, energy intake and energy retention of pigs at 25 kg and 65 kg under ambient temperatures from 18°C to 32°C. (A) Modelling the voluntary feed intake (dark curves) and comparison with models developed previously by Quiniou et al. [20] (grey curves).

(B) Modelling the metabolizable energy intake (dark curves) and comparison with models developed previously by Guo et al. [21] (grey curves). (C) Modelling the energy retention as protein. (D) Modelling the energy retention as lipid.

Figure 2

Test the goodness-of-fit of models developed in the current study. (A) Plot of predicted voluntary feed intake vs. actual voluntary feed intake. (B) Plot of predicted metabolizable energy intake vs. actual metabolizable energy intake. (C) Plot of predicted energy retention as protein vs. actual energy retention as protein. (D) Plot of predicted energy retention as lipid vs. actual energy retention as lipid. (E) Plot of predicted voluntary feed intake vs. voluntary feed intake residuals. (F) Plot of predicted metabolizable energy intake vs. metabolizable energy intake residuals. (G) Plot of predicted energy retention as protein vs. energy retention as protein residuals. (H) Plot of predicted energy retention as lipid vs. energy retention as lipid residuals.

Figure 3

Principle component analysis (PCA) score plot demonstrating the separation of the plasma samples from pigs at 25 kg kept under different ambient temperatures. Low: 18°C; Neutral: 23°C; High: 32°C. Each circle represents an individual plasma sample.

Figure 4

Identified compounds through metabolomics analysis with different intensities in plasma samples from pigs at 25 kg kept under different ambient temperatures. Low:18°C; Neutral: 23°C; High: 32°C.