The effects of heat stress and plane of nutrition on metabolism in growing pigs


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ABSTRACT: Heat stress (HS) jeopardizes pig health, reduces performance variables, and results in a fatter carcass. Whether HS directly or indirectly (via reduced feed intake) is responsible for the suboptimal production is not known. Crossbred gilts (n = 48; 35 ± 4 kg BW) were housed in constantly climate-controlled rooms in individual pens and exposed to 1) thermal-neutral (TN) conditions (20°C; 35% to 50% humidity) with ad libitum intake (n = 18), 2) HS conditions (35°C; 20% to 35% humidity) with ad libitum intake (n = 24), or 3) pair-fed [PF in TN conditions (PFTN), n = 6, to eliminate confounding effects of dissimilar feed intake (FI)]. Pigs in the TN and HS conditions were sacrificed at 1, 3, or 7 d of environmental exposure, whereas the PFTN pigs were sacrificed after 7 d of experimental conditions. Individual rectal temperature (Tr), skin temperature (Ts), respiration rates (RR), and FI were determined daily. Pigs exposed to HS had an increase (P < 0.01) in Tr (39.3°C vs. 40.8°C) and a doubling in RR (54 vs. 107 breaths per minute). Heat-stressed pigs had an immediate (d 1) decrease (47%; P < 0.05) in FI, and this magnitude of reduction continued through d 7; by design the nutrient intake pattern for the PFTN controls mirrored the HS group. By d 7, the TN and HS pigs gained 7.76 and 1.65 kg BW, respectively, whereas the PFTN pigs lost 2.47 kg BW. Plasma insulin was increased (49%; P < 0.05) in d 7 HS pigs compared with PFTN controls. Compared with TN and HS pigs, on d 7 PFTN pigs had increased plasma NEFA concentrations (110%; P < 0.05). Compared with TN and PFTN controls, on d 7 circulating Nτ-methylhistidine concentrations were increased (31%; P < 0.05) in HS pigs. In summary, despite similar nutrient intake, HS pigs gained more BW and had distinctly different postabsorptive bioenergetic variables compared with PFTN controls. Consequently, these heat-induced metabolic changes may in part explain the altered carcass phenotype observed in heat-stressed pigs.

Key words: heat stress, insulin, metabolism, pigs

INTRODUCTION

Animal agriculture is severely affected by heat stress (HS); the U.S. swine industry loses over $300 million annually, and global loses are in the billions (St-Pierre et al., 2003). Part of the economic distress originates in decreased carcass value (increased lipid and decreased protein content) and carcass processing problems (St-Pierre et al., 2003). The fact that pigs reared in HS conditions have reduced muscle mass and increased adipose tissue has been documented frequently over the past 40 yr (Close et al., 1971; Bridges et al., 1998; Collin et al., 2001).

The mechanisms responsible for the altered hierarchy in tissue accretion rates during HS are not clear. However, postabsorptive changes in nutrient partitioning in some heat-stressed models do not reflect normal metabolic modifications observed in thermal-neutral (TN) animals on a similar restricted plane of nutrition. For example, using a pair-feeding model (to distinguish between the direct and indirect effects of HS), we have demonstrated that despite marked reductions in nutrient intake and BW loss, heat-stressed ruminants have increased basal and stimulated circulating insulin concentrations (O’Brien et al., 2010; Wheelock et al., 2010).

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The altered insulin variables may also explain why heat-stressed ruminants do not mobilize adipose tissue triglycerides despite being in a hypercatabolic state (Rhoads et al., 2009; Shwartz et al., 2009; O’Brien et al., 2010).

Current study objectives were to metabolically differentiate between the direct and indirect effects (mediated by reduced nutrient intake) of an extensive thermal load on production parameters and postabsorptive metabolism in growing pigs. We hypothesized that heat directly (independent of reduced nutrient intake) affects pig performance and postabsorptive metabolism, and this altered energetic homeostasis may partially explain the phenotypic change observed (increased carcass lipid) in heat-stressed pigs.

MATERIALS AND METHODS

Iowa State University Institutional Animal Care and Use Committee approved all procedures involving animals.

Animals and Experimental Design

Female crossbred gilts (n = 48, 43 ± 4 kg BW; PIC C22/C29 × 337, Carthage Veterinary Service, Carthage, IL) were selected by BW and housed in individual pens (with individual feeders and waters) in 1 of 2 rooms (24 pens/room) at TN conditions (20°C ± 1°C; 35% to 50% relative humidity; Federation of Animal Sciences Societies, 2010). Animals were allowed to acclimate to their environment for 5 d. Pigs were then assigned to 1 of 3 environmental treatments: 1) TN conditions with ad libitum feed intake, 2) HS conditions (constant 35°C ± 1°C; 20% to 35% relative humidity) with ad libitum intake, or 3) TN conditions but pair fed (PFTN) to mirror the nutrient intake of the HS pigs. To evaluate the temporal response to environment, pigs in the TN (n = 18) and HS (n = 24) conditions were euthanized at d 1 (n = 6 HS and n = 6 TN), d 3 (n = 6 HS and n = 6 TN), and d 7 (n = 12 HS and n = 6 TN) postinitiation of environment treatment. The PFTN pigs (n = 6) were only euthanized at 7 d postinitiation of nutrient restriction.

After environmental initiation, reduced feed intake in the HS pigs was calculated daily on the basis of the percentage decrease from the average feed intake of each animal before HS, as we have previously described (Rhoads et al., 2009; Wheelock et al., 2010); the amount of feed offered to PFTN pigs was based on that percentage of decrease. The PFTN group lagged 1 d behind the 7-d HS pigs to calculate and implement feed intake reductions. Pair feeding was used to eliminate confounding effects of dissimilar nutrient intake. Individual animal as-fed intake was determined daily at 0800 h (ort DM was not determined). Pair-fed pigs were fed calculated amounts thrice daily at 0700, 1200, and 2000 h in an attempt to reduce large postprandial shifts in carbohydrate and lipid metabolism. Regardless of environmental treatment, all animals were fed the same diet throughout the duration of the experiment. All pigs were fed a standard swine grower diet consisting mainly of corn and soybean meal formulated to meet or exceed NRC (1998) requirements (Table 1).

The temperature and humidity of each room were continuously monitored by a data logger (Lascar model EL-USB-2-LCD, Erie, PA) that recorded environmental data in 30-min intervals. The ambient temperature of each room was controlled, but humidity was not governed. All pigs were monitored continuously for signs of distress (body temperature indices). Body temperature variables were obtained 3 times daily (0800, 1200, and 2000 h) and were condensed into daily averages for statistical purposes. Rectal temperatures (Ts) were recorded with a digital thermometer (Top Care, Waukegan, IL), and respiration rates (breaths/min) were determined visually with time measured by a stopwatch. Heat-stressed pigs were removed from the room and cooled with cool water (10 min) if rectal temperature exceeded 41.0°C (this occurred with 8 pigs). Carcass stored heat was calculated by Tr (°C) × specific heat of tissue (0.8°C) × BW (kg) as previously described (Silanikove, 2000). The ME required for maintenance (ME maintenance) was calculated according to the equation from NRC (1998): ME maintenance (Mcal/d) = 0.106 Mcal ME/d × BW0.75.

Body weights were recorded on all animals (0700 h) at the beginning of the experiment, at the beginning of

Table 1. Ingredients and chemical composition of diet (as-fed basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients</td>
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</tr>
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<tr>
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<td>20.65</td>
</tr>
<tr>
<td>Dried distillers grains (DDGS)</td>
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<tr>
<td>Vitamin and mineral mix1</td>
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<tr>
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</tr>
<tr>
<td>Threonine</td>
<td>0.03</td>
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<tr>
<td>Energy and nutrients</td>
<td></td>
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<td>DM</td>
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<tr>
<td>CP</td>
<td>17.51</td>
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<tr>
<td>Crude fat</td>
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</tr>
<tr>
<td>ME2, Mcal/kg</td>
<td>3.12</td>
</tr>
</tbody>
</table>

1Supplied per kilogram of diet: vitamin A, 8,364 IU; vitamin D3, 1,533 IU; vitamin E, 45 IU; vitamin K, 2.2 IU; choline, 6.5 mg; riboflavin, 4.2 mg; niacin, 21 mg; pantothenic acid, 17 mg; vitamin B12, 28 μg; biotin, 1.6 μg; folic acid, 0.0005 mg; Zn, 112 mg/kg as zinc sulfate and zinc oxide; Mn, 54 mg/kg as manganous oxide; Fe, 145 mg/kg as ferrous carbonate and ferrous sulfate; Cu, 20 mg/kg as copper chloride; I, 0.76 mg/kg as ethylenediamine dihydriodide; Se, 0.25 mg/kg as sodium selenite.

2Estimated using the NRC (1998) individual dietary ingredients.
the environmental treatment, and immediately preceding sacrifice. Jugular vein blood was obtained (10-mL BD Vacutainers (BD, Franklin Lakes, NJ) containing 143 units of sodium heparin) via venipuncture immediately before sacrifice (captive bolt technique followed by exsanguination). Blood was centrifuged at 1,300 × g for 15 min at 4°C to obtain plasma and was then stored at -20°C for later analysis.

**Metabolite and Hormone Assays**

One plasma aliquot was analyzed at the Iowa State Veterinary Diagnostic Laboratory for a routine blood panel [large animal complete panel consisting of blood urea nitrogen (BUN), creatinine, glucose, total protein, albumin, creatine kinase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transferase, calcium, phosphorus, sodium, potassium, chloride, triglycerides, cholesterol, and NEFA]. All plasma samples were analyzed on a Vitros 5.1 FS chemistry analyzer (Ortho Clinical Diagnostics, Rochester, NY), which simultaneously measures plasma samples in duplicate for all the aforementioned diagnostic tests.

Plasma samples were filtered at 10,000 × g for 10 min at 4°C using 10-kDa molecular weight cutoff filters (Pall Corp., Port Washington, NY). Filtered plasma was subjected to vaporized anoxic acid hydrolysis (Cohen et al., 1989). Plasma Nt-α-methylhistidine (NtMH) concentration was quantified with an HPLC method (PICO-Tag reverse-phase column; Waters, Milford, MA) as previously described and validated (Cohen et al., 1989). Plasma insulin concentration from pigs euthanized on d 7 were analyzed in duplicate using an ELISA kit solid phase 2-site enzyme immunoassay based on the sandwich technique (Merckia Porcine Insulin ELISA, ALPCO Diagnostics, Salem, NH) validated in our laboratory. The assay contains 5 calibrators as well as internal serum pools with low, intermediate, and high porcine insulin concentrations. The assay was conducted in 96-well microplates and read at 450 nm using a Synergy 4 microplate reader (Bio-Tek, Winooski, VT). Inter- and intra-assay CV for the plasma insulin assay were 4.3% and 4.1%, respectively.

**Analysis of Gene Expression**

Gene expression analysis was performed as described previously (Rhoads et al., 2011). Briefly, total RNA was extracted from skeletal muscle using the Trizol reagent according to the manufacturer’s protocol (Invitrogen, Carlsbad, CA). Total RNA was DNase treated (RNase-Free DNase set, Qiagen Inc., Valencia, CA) and purified by affinity chromatography (RNeasy, Qiagen Inc.) to remove potential genomic DNA contamination. The concentration of total RNA was determined by absorbance at 260 nm, and quality was verified by the Experion System (Bio-Rad Laboratories Inc., Hercules, CA). Total RNA was stored at -80°C until gene expression analysis.

Total RNA (2 μg) was reverse transcribed using the SuperScript First Strand Synthesis System for RT-PCR (Invitrogen). Primer sets for porcine heat shock protein 70 (HSP70) were designed using Primer Express Software (Applied Biosystems, Grand Island, NY) on the basis of published porcine nucleotide sequence (accession number NM_213766.1). Twenty-five microliter reactions were prepared according to the manufacturer’s instructions using the iQ SYBR Green Supermix (Bio-Rad Laboratories Inc.), 100 pM of each forward and reverse primer, and 10 ng of cDNA. Polymerase chain reaction quantification of each sample was performed in triplicate, and SYBR Green fluorescence was quantified with the iQ5 Real Time PCR Detection System (Bio-Rad Laboratories Inc.). Each assay plate contained negative controls and a standard curve (5 serial dilutions of a pool cDNA sample) to determine amplification efficiency of the respective primer pair. For each assay 40 PCR cycles were run, with each cycle consisting of 3 stages (50°C for 2 min, 95°C for 10 min, and 60°C for 1 min), and a dissociation curve was included to verify the amplification of a single PCR product. Analyses of amplification plots were performed with the iQ5 Optical System Software version 2.0 (Bio-Rad Laboratories Inc.).

**Analysis of Protein Expression**

Longissimus dorsi samples were homogenized, and proteins were extracted and analyzed for protein content using a commercially available assay kit (Pierce BCA microplate protein assay kit, Pierce, Rockford, IL) and frozen at ~80°C for later use. The lysis buffer consisted of 1% Triton X-100, 50 mM Heps, 150 mM NaCl, 10% glycerol, 50 mM EDTA, and 0.1% SDS with protease and phosphatase inhibitors (Na3VO4, leupeptin, aprotinin, pepstatin, Phenylmethylsulfonyl fluoride, NaF; Sigma-Aldrich, St. Louis, MO). To validate similar protein concentrations between samples, equivalent volumes of all samples were separated using 15% polyacrylamide gel (acrylamide:amide:N,N'-bis-methylene acrylamide = 100:1) consisting of 0.1% SDS (wt/vol), 0.05% Tetrathymethylenediamine (vol/vol), 0.05% ammonium persulfate (vol/vol), and 0.5 M Tris-HCL pH 8.8. Gels were then stained with Coomassie Brilliant Blue (100 mg brilliant blue/100 mL destain), followed by washing with destain (consisting of 40% methanol, 7% glacial acetic acid, and water) overnight (Sasse and Gallagher, 2009).
After load validation, muscle homogenates (50 μg) were separated by SDS (10%) polyacrylamide gel electrophoresis (SDS-PAGE) with the same recipe as mentioned above, and proteins were detected as previously described (Huff-Lonergan et al., 1996). Briefly, gels were run under reducing conditions and transferred to nitrocellulose membranes. Membranes were blocked for 1 h in 5% nonfat dry milk in Tris-Buffered Saline with Tween 20. Membranes were then blocked in primary antibody (mouse HSP70; Novus Biologicals, Littleton, CO) with 5 mL 5% BSA overnight. After blocking in primary antibody, membranes were washed 3 times for 10 min each with TBST (1X TBS, 0.1% Tween-20) and then incubated with secondary Horseradish peroxidase antibody (anti-mouse, Cell Signaling, Danvers, MA) for 1 h. Finally, membranes were washed 3 times for 10 min each with TBST. For detection, Supersignal West Pico Chemiluminescent Substrate was used (Thermoscientific, Waltham, MA), and membranes were incubated for 5 min before imaging. Membranes were imaged using FOTO Analyst Luminary/FX (Fotodyne Inc., Hartland, WI). Band densities were quantified by densitometry using TotalLab Quant (Total Lab, Newcastle upon Tyne, UK). Control samples were used on all gels, and bands were standardized to the density of the controls.

**Statistical Analysis**

All data were statistically analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC). Data are reported as least squares means and are considered significant if \( P < 0.05 \). Data were evaluated using 2 distinct models. For daily measurements (body temperatures, respiration rates, and feed intake) the respective variable of each animal (from all 3 treatments) was analyzed using repeated measures with an autoregressive covariance structure and day as the repeated effect. The model included environment, day, and the interaction and prethermal treatment as a covariate. If the measurement was obtained only once (blood and molecular data), the model contained treatment (just TN and HS), day (1, 3, or 7), and the treatment by day interaction and was not analyzed by repeated measures and did not contain a covariate. Because the PFTN pigs were only euthanized on d 7, a separate analysis only tested differences between all 3 environments (TN, PFTN, HS) on just d 7.

**RESULTS**

Irrespective of day or time of day, pigs exposed to HS had an overall increase \( (P < 0.01) \) in rectal temperature (39.3 vs. 40.8°C) compared with TN pigs, and this increase was maintained throughout the duration of the experiment (Fig. 1A). Rectal temperatures in PFTN pigs were decreased compared with TN (0.44°C; \( P < 0.01 \)) and HS (1.94°C; \( P < 0.01 \)) pigs by d 1, and this difference in body temperature was maintained throughout all 7 d. Independent of time of day, compared with TN and PFTN pigs, HS animals had an immediate increase \( (P < 0.05) \) in respiration rate on d 1 [49 vs. 136 breaths per minute (bpm)]. Respiration rates decreased \( (P < 0.05) \) in HS pigs on d 2 and 3 but remained elevated \( (P < 0.05) \) compared with TN and PFTN pigs (55 vs. 115 bpm) throughout d 7 (Fig. 1B). Respiration rates did not differ \( (P > 0.10) \) between PFTN and TN pigs.

Thermal-neutral pigs consumed 1.96 kg/d of feed, and this amount did not differ \( (P > 0.10) \) over time (Fig. 1C). Compared with TN pigs, HS pigs had an immediate reduction (47%; \( P < 0.05 \)) in feed intake by d 1, and this extent of decrease continued through d 7. By design, the nutrient intake pattern of PFTN pigs mir-

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**Figure 1.** Effects of ad libitum feed intake in thermal-neutral conditions (TN; 20°C), ad libitum feed intake in constant heat stress (HS; 35°C) conditions, and pair feeding in thermal-neutral conditions (PFTN) on the temporal changes in (A) rectal temperatures, (B) respiration rates (bpm = breaths per minute), and (C) feed intake in growing pigs.
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ored that of the HS group. During the experiment, TN pigs had an ADG of 1.14 kg/d and had BW gains of 1.27, 3.12, and 7.76 kg at 1, 3, and 7 d, respectively (Fig. 2A). Heat-stressed pigs lost 2.7 kg of BW at 1 d post-HS initiation but had gained 0.03 and 1.65 kg BW by d 3 and 7, respectively. By d 7, the PFTN pigs had lost 2.47 kg of BW and weighed 4.12 and 10.23 kg less (P < 0.01) than the HS and TN pigs, respectively (Fig. 2B).

There was an overall increase (58%; P < 0.05) in LM HSP70 mRNA abundance in HS pigs compared with TN controls (Table 2). On d 7, the LM HSP70 gene expression decreased 51% (P < 0.05) in PFTN controls compared with TN and HS pigs (Table 2). There was an environment by day interaction (P = 0.05) for LM HSP70 protein abundance, as it was increased 151% in HS pigs at d 1 and temporally decreased (but was still elevated 75% compared with TN pigs) by d 7 (Fig. 3A). On d 7, LM HSP70 expression was 21% lower (P < 0.05) in PFTN compared TN pigs and was 2.2-fold greater (P < 0.05) in HS pigs compared with PFTN pigs (Fig. 3B).

On d 7, plasma insulin values were least in PFTN pigs and incrementally increased (P < 0.05) in HS and TN pigs (0.06, 0.12, and 0.17 ng/mL, respectively; Fig. 4A). There was an environment by day interaction (P < 0.05) in circulating NEFA content, as HS pigs had an immediate (d 1) and marked (67%; P < 0.05; Table 2) increase in blood NEFA concentrations compared with TN controls but similar NEFA concentrations as TN pigs on d 3 and 7 (Table 2). By d 7, circulating NEFA concentrations in PFTN pigs were increased 137% (P < 0.05) compared with TN and HS pigs (Fig. 4B). Overall, HS pigs had reduced (7.1%; P < 0.05) plasma glucose concentrations compared with TN controls, and this is primarily due to differences (11.3 mg/dL) at d 3 and 7 (Table 2). At d 7, analysis of all 3 environments indicates no treatment differences (P > 0.37; Fig. 4C) in plasma glucose concentrations (109 mg/dL). There was an environment by day interaction in BUN concentrations as circulating BUN increased 35% (P < 0.05) on d 1 in HS pigs compared with TN controls, but HS pigs had reduced (18%; P < 0.05) BUN concentrations compared with TN controls on d 3 and 7 (Table 2). On d 7, BUN concentrations in PFTN pigs were similar (P > 0.37) to both TN and HS pigs. There was an environment by day interaction in plasma cholesterol, as cholesterol concentrations increased (P < 0.05) in HS pigs compared with TN controls at d 1 (25%) and d 3 (22%) but were similar to TN and PFTN pigs by d 7 (Table 2). There were no environmental differences (P < 0.11) in plasma triglyceride concentrations (Table 2).

Overall, HS had increased (16%; P = 0.05) circulating NtMH compared with TN pigs, and d 7 analysis indicates that HS pigs had elevated plasma NtMH (31%; P < 0.05) compared with both the TN and PFTN controls (Table 2). Heat-stressed pigs had an immediate (d 1) and sustained increase (50%; P < 0.05; Table 2) in plasma creatinine compared with both TN and PFTN controls (Fig. 4D). There were no treatment differences in plasma creatine kinase concentrations (P > 0.20; Table 2). Plasma total protein increased (7.4%; P < 0.01) in HS compared with TN pigs and was incrementally increased (P < 0.05) in HS and PFTN compared with TN pigs on d 7 (6.3a, 6.8b, and 7.4c g/dL, respectively). There tended (P = 0.10) to be an environment by day interaction in plasma albumin content as it was increased (12%) in HS compared with TN pigs on d 1 but was comparable to TN control concentrations on d 3 and 7 (Table 2). On d 7, plasma albumin concentration was increased (22%; P < 0.01) in PFTN compared with both TN and HS pigs (Table 2). There was an overall treatment effect (P < 0.01) in plasma alkaline phosphatase concentration between TN and HS pigs, and there tended (P = 0.11) to be an environment by day interaction as it was decreased (14% and 50%) in HS compared with TN pigs on d 1 and 7, respectively (Table 2). On d 7, HS pigs had a 51% reduction (P < 0.01) in plasma alkaline phosphatase content compared with both the TN and PFTN controls (Table 2). Overall...

Figure 2. (A) Effects of ad libitum feed intake in thermal-neutral conditions (TN; 20°C) and ad libitum feed intake in constant heat stress (HS; 35°C) conditions on the temporal changes in BW and (B) effects of TN, HS, and pair feeding in thermal-neutral conditions (PFTN) on BW change on d 7 in growing pigs. Bars with different letters (a,b,c,d,e) differ at P < 0.05.
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plasma γ-glutamyl transferase tended \((P = 0.07)\) to be increased (13%) in HS compared with TN pigs, but no differences were detected between TN, HS, and PFTN pigs on d 7 (Table 2). There were no environmental differences \((P > 0.15)\) observed in plasma aspartate amino transferase concentrations (Table 2).

Plasma bicarbonate concentrations were overall \((P < 0.01)\) decreased (10.5%) in HS compared with TN controls, but no treatment differences \((P > 0.47)\) were detected between TN, HS, and PFTN controls on d 7 (Table 3). Overall, plasma calcium concentrations were decreased \((P < 0.01; 5.5\%)\) in HS compared with TN pigs, but on d 7 plasma calcium concentrations in PFTN pigs did not differ from either TN or HS pigs (Table 3). Overall, plasma potassium content tended \((P = 0.07)\) to be reduced (9.5%) in HS compared with TN pigs, but no treatment differences were detected between TN, HS, and PFTN pigs on d 7 (Table 3). There was a treatment by day interaction \((P < 0.01)\) in plasma sodium content as it was increased on d 1 in HS pigs but decreased on d 7 compared with TN controls (Table 3). There was an environment by day interaction \((P < 0.01)\) in plasma chloride concentrations as it was increased on d 1 and 3 in HS compared with TN pigs but was not different from TN controls on d 7 (Table 3). There were no treatment differences \((P > 0.25)\) detected between TN, HS, and PFTN pigs in plasma chloride concentrations on d 7 (Table 3). There was a treatment by day interaction \((P < 0.01)\) on plasma phosphorus concentrations as they were increased 15% in HS compared with TN pigs on d 1 but were decreased 17% in HS pigs compared with TN controls on d 7 (Table 3). By d 7, HS pigs had reduced (15%; \(P < 0.01)\) plasma phosphorus concentrations compared with both TN and PFTN controls (Table 3).

### DISCUSSION

Heat stress reduces domestic animal production parameters and negatively affects the global agriculture economy. The heat-induced financial burden is due to increased morbidity, mortality (especially in market-weight hogs), suboptimal growth, inconsistent market weights, inefficient nutrient use, poor sow performance,

<table>
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<th>Variable</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>SEM</th>
<th>P-value</th>
<th>Day of experiment</th>
<th>SEM</th>
<th>P-value</th>
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<tbody>
<tr>
<td>LM HSP70(^4), ΔCT</td>
<td>5.98</td>
<td>13.20</td>
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<td>0.27(^b)</td>
<td>0.06(^a)</td>
<td>0.09(^a)</td>
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<td>13.8(^a)</td>
<td>11.2(^b)</td>
<td>8.4(^cd)</td>
<td>10.6(^bc)</td>
<td>7.3(^d)</td>
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<td>CK, IU/L</td>
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<td>47.8</td>
<td>37.0</td>
<td>53.2</td>
<td>46.8</td>
<td>47.8</td>
<td>3.9</td>
<td>0.07</td>
</tr>
<tr>
<td>AST, IU/L</td>
<td>53.5</td>
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<td>76.0</td>
<td>79.5</td>
<td>54.0</td>
<td>50.8</td>
<td>11.7</td>
<td>0.95</td>
</tr>
</tbody>
</table>

\(^a\) or \(^b\) \(P < 0.05\).

1. Thermal neutral.
2. Treatment × day interaction.
3. Pair-fed thermal neutral.
4. LM heat shock protein 70 gene expression.
5. Triglycerides.
7. \(\tau\)-methylhistidine.
8. Creatine kinase.
10. Gamma-glutamyl transferase.
11. Aspartate amino transferase.
decreased carcass value (increased lipid and decreased protein content), and carcass processing problems (St-Pierre et al., 2003). Determining how HS detrimentally affects energetic homeostasis and nutrient partitioning in pigs may allow for future nutritional or pharmaceutical interventions to ameliorate the deleterious effects of increased environmental temperatures. An immediate effect of HS is a decrease in feed intake, and this reduced appetite is presumably a strategy to minimize metabolic heat production. Caloric restriction has obvious effects on growth and metabolism; therefore, to differentiate between the direct and indirect effects (mediated by reduced nutrient consumption) of HS, we used a TN ad libitum model and a PFTN model to eliminate the confounding effects of dissimilar feed intake.

Our HS protocol resulted in marked hyperthermia as all body temperature indices in the HS pigs were elevated compared with both TN and PFTN controls. The HS regimen was constant, and the lack of a cyclical ambient temperature pattern prevented the pigs from having a normal diurnal Tr pattern. Despite the constant heat load, both LM HSP70 (HSP70 plays a hyperheightened role in cryoprotection; Volloch and Rits, 1999) gene expression and protein abundance decreased with time, and this temporal pattern likely represents intracellular acclimation. The PFTN pigs had a reduced body temperature (0.6°C) compared with the TN controls, and this presumably denotes a reduced heat increment of feeding. Muscle HSP70 must be incredibly sensitive to small fluctuations in body temperature as the LM HSP70 gene expression and protein abundance were lower in the PFTN pigs compared with the TN controls. On a thermal energy basis, the PFTN pigs had, on average, 195 kcal less total heat stored than TN pigs, and this represents only 6% of the difference in energy intake (TN pigs consumed ~6.33 Mcal ME/d, but the HS and PFTN pigs consumed ~3.20 Mcal ME/d).

Heat stress caused an immediate decrease in feed intake that remained less (46%) than TN controls throughout the experiment. By design, PFTN pigs had a pattern of reduced feed intake similar to the HS group. Decreased feed intake during HS was expected as this is a highly conserved response among species (Collin et al., 2001; Baumgard and Rhoads, 2012). Heat-stressed pigs lost almost 3 kg of BW within 24 h but had gained 1.65 kg of BW by d 7. Pair-fed TN controls were not weighed on d 1 or 3, so we are unable to determine how much of the BW loss at d 1 in the HS pigs is due to reduced feed intake. However, nutrient intake only decreased 0.92 kg within the first day of HS, so clearly, other contributing factors account for the additional 2 kg of immediate BW loss in the HS pigs, and we speculate this is a combination of water and gastrointestinal tract contents.

In contrast to our expectations, on d 7 the HS pigs had actually gained BW (1.65 kg), whereas the PFTN controls lost BW (2.47 kg), and we have confirmed this BW differential in a similar experiment (Sanz-Fernandez et al., 2012). The fact that PFTN controls lost BW is unexpected as they were consuming almost 2.00 Mcal/d more than their calculated maintenance costs (~1.50 Mcal/d). The classic definition of energy balance is the gain or loss of BW, so we are obviously not accounting for some aspect of bioenergetics in the PFTN pigs. Regardless, although the chemical composition of that BW difference between PFTN and HS pigs is unknown, the literature suggests that pigs and other species gain more adipose tissue than energetically predicted during heat stress (Heroux and Gridgeman, 1958; Yunianto et al., 1997). If the BW difference between HS and PFTN pigs were all adipose tissue, the 4.12 kg would equal more than 37 Mcal of energy, but this is 13 Mcal more energy than the PFTN and HS pigs consumed during the entire 7 d experiment. Clearly, the 4.12 kg is not all body fat and likely represents a combination of 1) decreased basal metabolic rate in HS pigs, 2) marked

Figure 3. (A) Effects of ad libitum feed intake in thermal-neutral conditions (TN; 20°C) and ad libitum feed intake in heat stress (HS; 35°C) conditions on the temporal change in longissimus dorsi (LD) heat shock protein 70 (HSP 70) expression and (B) effects of TN, HS, and pair feeding in thermal-neutral conditions (PFTN) on change in LD HSP 70 on d 7 in growing pigs. Bars with different letters (a,b,c) differ at \( P < 0.05 \).
differences in adipose and lean tissue accretion and catabolic rates, and 3) an increase in activity energy expenditure in the PFTN pigs (the PFTN pigs were visibly anxious). Previous results suggest that reduced feed intake explains the decreased growth in heat-stressed pigs (Safranski et al., 1997; Collin et al., 2001) and cattle (O’Brien et al., 2010), but our data indicate that PFTN controls actually lost considerably more BW than HS pigs. This is in stark contrast to the lactation model demonstrating that milk yield from HS cows is reduced more than 2-fold compared with PFTN controls (Rhoads et al., 2009; Wheelock et al., 2010).

Animals on a reduced plane of nutrition are typically hypoinsulinemic, and this is a highly conserved postabsorptive effect among species (Baumgard and Rhoads, 2012). Our PFTN model had reduced plasma insulin compared with HS and TN pigs on d 7. Both HS and PFTN pigs were nutrient restricted (~50%), and although both had decreased circulating insulin compared with the TN pigs, the HS animals had 100% greater insulin concentrations than the PFTN pigs. This is unusual as they were both on an equivalent plane of inadequate/suboptimal nutrition. In addition, HS animals are thought to be in a catabolic state, and epinephrine and cortisol both typically inhibit pancreatic insulin secretion (Doi et al., 1982). This peculiar insulin response agrees with a previous malignant hyperthermic pig model (Hall et al., 1980) as well as data for heat-stressed rodents (Tolinska et al., 1987), growing steers (O’Brien et al., 2010), and lactating cows (Wheelock et al., 2010). Increased insulin action appears to be an important survival adaptation to elevated temperatures as diabetic rats (Niu et al., 2003) and humans (Bouchama et al., 2007) are more susceptible to heat stress. Reasons for hyperinsulinemia during HS are not clearly understood but likely include the key role of insulin in activating and up-regulating heat shock proteins (Li et al., 2006).

Heat stress decreased circulating glucose concentrations compared with TN pigs, and this appears to be mainly due to decreased feed intake as no differences between HS and PFTN treatments were detected on d 7. Our current results are similar to findings in heat-stressed pigs (Becker et al., 1992) and cows (Ronchi et al., 1999; Shwartz et al., 2009). In contrast to our results, previous studies indicated an increase in blood glucose concentrations in poultry (Bobek et al., 1997), rabbits (Marder et al., 1990), and pigs (Prunier et al., 1997). Reasons for the discrepancies in blood glucose (and other measures in the current study) in a myriad of studies may be due to the severity (both extent and acute vs. chronic) of the heat load, physiological state, and nutritional status.

Undernourished growing animals alter metabolism and mobilize adipose tissue, and this is a classic glucose-
sparing mechanism to prioritize skeletal protein accretion. As expected, after 7 d the PFTN pigs had increased plasma NEFA concentrations. However, HS pigs only had increased plasma NEFA concentrations at d 1 but had similar NEFA concentrations as TN pigs at d 3 and 7. This temporal pattern is similar to what is observed in heat-stressed chickens (Bobek et al., 1997), and the marked increase in NEFA on d 1 suggests that adipose tissue mobilization may have partially contributed to the acute loss of BW in the HS pigs (Fig. 2). Reasons for the immediate increase in NEFA and subsequent decline are ill defined but may correlate to the temporal pattern of stress hormones (i.e., cortisol, catecholamines), which have been shown to increase during acute heat stress (Beede and Collier, 1986) but have not been thoroughly characterized during chronic heat stress (especially in the pig model). Regardless, this eventual decrease in circulating NEFA also agrees with previous research demonstrating decreased NEFA concentrations in malignant hyperthermic pigs (Hall et al., 1980), rodents (Sano et al., 1983), and cattle (Rhoads et al., 2009; Shwartz et al., 2009). Despite agreeing with HS literature, this is somewhat unexpected as heat-stressed animals are on a lowered plane of nutrition. Insulin is a potent lipolytic inhibitor, and the aforementioned increase in basal insulin may explain the lack of a NEFA response during heat stress (Baumgard and Rhoads, 2012). Reducing the amount and/or activity of skeletal muscle lipoprotein lipase [the muscle enzyme that hydrolyzes very low density lipoproteins (VLDL) and chylomicron triglycerides] is one logical mechanism that would support the aforementioned metabolic switch, and this would likely increase circulating concentrations of both triglycerides and chylomicrons (main components of both VLDL and chylomicrons).

Heat stress likely affected postabsorptive protein metabolism, as illustrated by gross changes in BW, and this agrees with how environmentally induced hyperthermia affects a variety of species (Schmidt and Widdowson, 1967; Close et al., 1971; Lu et al., 2007). The phenotypic change in BW is corroborated by increased circulating concentrations of NtMH, a reliable marker of skeletal muscle catabolism (Yoshizawa et al., 1997). Although the lack of an environment effect on creatine kinase appears to contradict the NtMH data, the acute increase in BUN and chronic and sustained elevation in circulating creatinine also support the idea that severe HS stimulates muscle breakdown. Interpreting changes in creatine kinase, BUN, and creatinine is difficult because multiple insults can affect their concentration and their origin is frequently ambiguous. Regardless, on the basis of the NtMH it is clear that skeletal muscle catabolism is increased during HS, and numerous studies have reported increased plasma markers of muscle breakdown in a variety of heat-stressed species (Hall et al., 1980; Marder et al., 1990; Yunianto et al., 1997; Wheelock et al., 2010). Despite the apparent muscle catabolism, skeletal muscle insulin responsiveness remains unaltered during hyperthermia in lactating dairy cows (Cole et al., 2011). Such HS effects on muscle protein metabolism are paradoxical as insulin is a potent antiproteolytic and anabolic hormone (Allen, 1988). Identifying why skeletal muscle proteolysis occurs in heat-stressed animals despite hyperinsulinemia compared with their bioenergetics thermal-neutral counterparts is of obvious interest.

Table 3. Effects of heat stress (HS) on plasma variables in growing pigs

<table>
<thead>
<tr>
<th>Variable</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCO₃⁻, mEq/L</td>
<td>30.5</td>
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<td>31.0</td>
<td>28.0</td>
<td>28.3</td>
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<td>0.53</td>
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<td>Potassium, mg/dL</td>
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<td>0.07</td>
<td>0.76</td>
<td>0.79</td>
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<td>Sodium, mg/dL</td>
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<td>142c</td>
<td>139ab</td>
<td>139b</td>
<td>141bc</td>
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<td>Chloride, mEq/L</td>
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<td>105b</td>
<td>99a</td>
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<td>102ab</td>
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<td>P, mg/dL</td>
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<td>9.3c</td>
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<td>7.3a</td>
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</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>TN¹ HS</th>
<th>TN¹ HS</th>
<th>TN¹ HS</th>
<th>Trt</th>
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<tr>
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<td>7</td>
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<tr>
<td>P</td>
<td>TN PFTN</td>
<td>HS</td>
<td>SEM</td>
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</tbody>
</table>

¹Thermal neutral.
²Treatment × day interaction.
³Pair-fed thermal neutral.
⁴Bicarbonate.
Conclusions

Heat stress markedly slows BW gain in growing pigs, but despite similar nutrient intake, HS pigs gained more BW and had distinctly different postabsorptive bioenergetic variables compared with PFTN controls. Perhaps the biggest metabolic difference between heat-stressed and nutrient-restricted TN pigs is the increase in circulating basal insulin concentrations. Further, there appear to be differences between muscle and adipose tissues responsiveness (resistant and sensitive, respectively) to heat-stress-induced increased basal insulin concentrations as heat-stressed pigs do not mobilize adipose tissue triglycerides but increase skeletal muscle proteolysis. These unique changes in postabsorptive lipid and protein metabolism likely explain why heat-stressed pigs (and other species) deaminate lean tissue and accumulate more carcass lipid than what is bioenergetically expected.

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