

Title: Mitigating the negative effects of *in utero* heat stress on piglet welfare following weaning and transport – **NPB #20-094**

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Date Submitted: February 27, 2020

Industry Summary:

In utero heat stress negatively impacts swine health and productivity throughout their entire lifespan. Negative effects range from decreased growth performance and meat quality to reduced reproductive efficiency and greater stress and disease susceptibility. The decrease in growth performance and increased stress response of *in utero* heat-stressed pigs may be partially due to increased energy requirements compared to *in utero* thermoneutral counterparts. Therefore, the experimental objective was to determine whether providing newly weaned and transported *in utero* heat-stressed pigs with higher energy nutrient dense diets would improve growth performance and decrease stress during the nursery phase of growth. We determined that increasing the energy content of the diet did not improve growth performance of the *in utero* heat-stressed pigs as average daily body weight gain was reduced overall when compared to *in utero* thermoneutral controls. This response was likely due to the fact that pigs provided the nutrient dense diet voluntarily reduced their feed intake so that total energy consumed was similar to that of the control diet fed pigs. The decrease in body weight gain for the *in utero* heat-stressed pigs was accompanied by metabolic alterations that prevented them from mobilizing energy reserves following weaning and transport when compared to *in utero* thermoneutral pigs. Overall, these data indicate that *in utero* heat stress is detrimental to future pig growth performance and health, especially during times of increased stress such as weaning and transport. Furthermore, the negative impact on growth performance is not rescued by the provision of more nutrient dense diets.

Keywords: *in utero* heat stress, mitigation, pigs, transport, weaning,

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

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Scientific Abstract:

In utero heat stress (**IUHS**) increases pig energy requirements during postnatal life, and this may compound weaning and transport stress. Therefore, the study objective was to mitigate the negative effects of IUHS on piglet growth performance and welfare following weaning and transport through the provision of a nutrient dense (**ND**) nursery diet formulated to meet the greater energy requirements of IUHS pigs. Twenty-four gilts were bred and exposed to thermoneutral (**TN**; $n = 12$; $17.5 \pm 2.1^\circ\text{C}$) or heat stress (**HS**; $n = 12$; cycling 26°C to 36°C) conditions for the first half of gestation (d 6 to 59) and then TN conditions ($20.9 \pm 2.3^\circ\text{C}$) for the remainder of gestation. At weaning (16.2 ± 0.4 d), mixed-sex piglets ($N = 160$; 4.78 ± 0.15 kg BW) were transported (loading + transport + unloading) for 11 h 40 min from Columbia, MO to West Lafayette, IN. Piglets were blocked into pens ($n = 4$ pigs/pen) by *in utero* and dietary treatments: IUTN + C ($n = 10$ pens), IUTN + ND ($n = 10$ pens), IUHS + C ($n = 10$ pens), IUHS + ND ($n = 10$ pens). Treatment diets were fed from d 1 to 14 post-weaning and transport (**Period 1**), and from d 15 to 35 post-weaning and transport the C diet was fed to all pigs (**Period 2**). Production measures were taken in 7 d intervals to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), average daily energy intake (**ADEI**), gain:feed, and gain:energy intake. Blood samples were collected prior to transport (**Pre-T**), immediately following transport (**Post-T**), and on d 2, 7, 14, 28, and 35 post-weaning and transport to analyze glucose, insulin, cortisol, and non-esterified fatty acids (**NEFA**). Behavior was assessed through video-recording on d 3, 5, 8, 11, and 13 post-weaning and transport. Data were analyzed using PROC GLIMMIX in SAS 9.4. In Period 1, ADG was reduced ($P = 0.03$; 10.9 g/d) in IUHS vs. IUTN pigs. Pigs fed ND diets had reduced ADFI ($P = 0.02$; 8.9%) compared to C diet fed pigs during Period 1, which resulted in similar ADEI ($P = 0.24$; $1,115 \pm 46$ kcal/d). During transport, cortisol was decreased ($P = 0.03$; 25.8%) in IUHS vs. IUTN pigs. On d 2, glucose was decreased ($P = 0.01$; 13.5 ng/mL) in IUHS vs. IUTN pigs. No *in utero* treatment-related behavioral differences were observed, but lying behavior was reduced ($P = 0.03$; 4.4%) and standing behavior was increased ($P = 0.04$; 4.0%) in ND vs. C pigs. In summary, IUHS reduced growth performance in pigs following weaning and transport and providing a ND diet did not rescue lost performance.

Introduction:

Heat stress (**HS**) negatively impacts animal welfare and productivity and is of increasing concern for the U.S. swine industry. Heat stress causes skin vasodilation and increased respiration rates in pigs (Johnson et al., 2015a), which are sufficient mechanisms to combat mild HS. However, severe HS compromises pigs' ability to maintain eutheria and results in elevated core body temperature (Renaudeau et al., 2008), and increases circulating stress hormones (Johnson et al., 2018). In addition to the direct effects of HS during postnatal life, offspring from HS-compromised gestating sows display a variety of negative postnatal phenotypes that are attributed to *in utero* heat stress (**IUHS**; Johnson and Baumgard, 2019). Immediate effects of IUHS on offspring include reduced birth and placental weights (Lucy et al., 2012) along with decreased survival as compared to offspring of sows gestated under *in utero* thermoneutral conditions (**IUTN**; Omtvedt et al. 1971). Furthermore, long-term effects of IUHS have been observed and include reduced growth performance (Johnson et al., 2015b), altered post-absorptive metabolism (Boddicker et al., 2014; Byrd et al., 2019), increased maintenance costs (Chapel et al., 2017) and core body temperature set-point during the growing phase (Johnson et al., 2015c), and altered behavior following weaning and transport (Byrd et al., 2017). Combined,

IUHS negatively affects pig performance and may contribute to substandard animal welfare outcomes.

In utero heat-stressed piglets are metabolically inflexible in response to decreased energy availability and do not reduce insulin or mobilize adipose tissue during times of decreased blood glucose (i.e., fasting; Byrd et al., 2019). Because IUHS increases fasting heat production (**FHP**) in pigs during postnatal life (Chapel et al., 2017), an inhibition of mechanisms meant to mobilize energy reserves to meet the metabolic requirements of the pig may result in decreased growth performance and poor welfare. It has been hypothesized that IUHS pigs perform more stress behaviors following weaning and transport when compared to IUTN counterparts due to the aforementioned metabolic inflexibility (Byrd et al., 2019). Therefore, the study objective was to improve energy balance and growth performance and reduce the stress response of IUHS pigs following weaning and transport through the provision of a more nutrient-dense (**ND**) diet that met the increased metabolic requirements of IUHS pigs. We hypothesized that IUHS pigs would have reduced performance and an increased stress response relative to IUTN pigs due to greater maintenance costs, and that providing a ND diet during the first 14-d postweaning and transport would mitigate this negative outcome.

Objectives:

- 1.) To evaluate the impact of *in utero* heat stress on the stress response and performance of piglets following weaning and transport in a production environment.
- 2.) To mitigate the negative effects of *in utero* heat stress on piglet welfare and performance by increasing the energy density of the diet and improving energy balance following weaning and transport stress.

Materials & Methods:

Gestation to weaning

The University of Missouri Animal Care and Use Committee approved all procedures involving pregnant gilts and their piglets (protocol # 9340) and animal care and use standards were based upon the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010). Twenty-four first parity gilts (Landrace x Large White) were bred to a Duroc sire and then housed in the Brody Environmental Chambers at the University of Missouri. Pregnant gilts were blocked by body weight (**BW**) and assigned to either a thermoneutral [**TN**; n = 12; 17.5 ± 2.1°C; 70.2 ± 8.8% relative humidity (**RH**)] or HS chamber (n = 12). Heat-stressed pregnant gilts were maintained under TN conditions (17.0 ± 0.1°C; 79.2 ± 10.1% RH) for 5 d following artificial insemination. From d 6 to d 10 post-artificial insemination, cyclic HS temperatures of 26.3 ± 3.0°C nighttime to 31.4 ± 2.9 °C daytime and 61.2 ± 21.2% RH were applied to all HS pregnant gilts. From d 11 to d 59 post-artificial insemination, cyclic HS temperatures of 28.4 ± 0.2°C nighttime to 35.8 ± 0.2 °C daytime and 80.9 ± 6.0% RH were applied to all HS pregnant gilts. The HS treatment was only applied during the first half of gestation for HS pregnant gilts as early pregnancy is when most genetic imprinting occurs (Reik, 2007). Furthermore, exposing pregnant gilts to HS during the first half of gestation was done to mimic a late June/early July breeding [i.e., HS during early gestation (mid- to late- summer) and TN during late gestation (early to mid- fall) to better represent what may actually be occurring in commercial production systems in the U.S. Of the

24 first parity gilts, 9 were confirmed pregnant from the TN treatment and 12 were confirmed pregnant from the HS treatment. Therefore, only data from the gilts that were confirmed pregnant were used in the study. Respiration rate (**RR**), rectal temperature (**T_R**), shoulder skin temperatures (**T_{shoulder}**), and ear skin temperatures (**T_{ear}**) were measured in 2 d intervals on all gestating sows from d 0 to d 59 post-artificial insemination at 0800 and 1500 h. Respiration rate was measured by counting flank movements over a 1 min interval to determine breaths per minute (**bpm**). Rectal temperature was measured using a calibrated, lubricated YSI probe (Model 400, YSI Inc., Yellow Springs, OH; accuracy $\pm 0.1^\circ\text{C}$) attached to a thermistor thermometer (Model 8110-20; Cole-Parmer Instruments, Chicago, IL). Shoulder skin and **T_{ear}** temperatures were measured using an infrared thermometer (Raytek Raynger ST, Raytek, Everett, WA, accuracy $\pm 0.1^\circ\text{C}$). Following the 59-d thermal treatment period, all pregnant gilts were exposed to the same TN conditions ($20.9 \pm 2.3^\circ\text{C}$; $63.6 \pm 15.6\%$ RH) for the remainder of gestation (d 60 to farrowing). All pregnant gilt diets were formulated to meet or exceed nutrient requirements (NRC, 2012) and consisted mainly of corn and soybean meal. All pregnant gilts were limit fed throughout gestation (2.0 kg/d) to prevent maternal weight gain per commercial swine industry standards (NRC, 2012) and all pregnant gilts finished their daily allotment, regardless of thermal treatment. From farrowing to weaning (16.0 ± 1.1 d of age), production data were collected for individual gilts and their litters to include gestation length, live born, still born, number of mummies, and number of weaned pigs. Furthermore, anogenital distance was also recorded for all piglets at processing. During the farrowing to weaning phase, all litters were exposed to the same environmental conditions as recommended by the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (26 to 32°C with heat lamps; Federation of Animal Sciences Societies, 2010).

Transportation

At weaning, all piglets ($N = 233$) were removed from the dams and placed into a gooseneck livestock trailer (2.06×6.05 m; Featherlite Trailers, Cresco, IA). Piglets were provided 0.06 m^2 of space/pig as recommended by the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010). All piglets were then transported from Columbia, Missouri to the Purdue University Swine Farm in West Lafayette, IN and then unloaded from the trailer, individually weighed, and placed into pens. Feed and water were withheld throughout the entire transport process based on normal production practices. Two data loggers (Hobo; accuracy $\pm 0.2^\circ\text{C}$; data logger temperature/RH; Onset; Bourne, MA) were placed, evenly spaced, inside the trailer to record ambient temperature (**T_A**) and RH in 5 min intervals. During transport, the **T_A** and RH within the trailer was ($7.1 \pm 4.2^\circ\text{C}$; $55.9 \pm 18.7\%$ RH). Wood shavings and straw were spread along the floor of the trailer to provide insulation and bedding during transport and trailer openings were adjusted based on **T_A** (National Pork Board, 2015). Total transport time (loading, transport, unloading) took 11 h 40 min and covered 612 km where 39% of the time was on two-lane roads and 61% of the time was on four-lane roads.

Post-transportation and nursery phase

The Purdue Animal Care and Use Committee approved all postnatal animal procedures (protocol #1806001756) and animal care and use standards were based upon *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010). Upon arrival to the Purdue University Swine Farm, piglets were group-housed

in 40 mixed-sex pens at 4 pigs/pen (0.21 m²/pig; mixed sex pigs) and maintained in TN conditions based on pig age (29.0 ± 1.3°C; 22.5 ± 5.2% RH) until 5 wk of age. The remaining piglets (N = 73) were considered off trial and were utilized in subsequent experiments. Piglets were assigned to one of two dietary treatments [ND diet or control (C) diet] balanced by *in utero* treatment. Treatment diets were fed for 14 d in two phases and treatment combinations were as follows: IUTN + C (n = 10 pens), IUTN + ND (n = 10 pens), IUHS + C (n = 10 pens), IUHS + ND (n = 10 pens). Control diets were formulated to meet or exceed nutrient requirements based on piglet age (NRC, 2012) and ND diets were formulated to provide 12% more net energy (NE)/kg of feed (maintaining Lysine:NE ratios) than recommended by the NRC (Table 1). This was done in order to meet the previously described increase in maintenance requirements of IUHS pigs (Chapel et al. 2017). This 14-d phase will be referred to as Period 1 throughout the remainder of this paper.

Following the 14-d dietary treatment period, all pigs were fed a common diet to meet or exceed nutrient requirements based on piglet age (NRC, 2012) from d 14 to the end of the nursery period (d 35; Table 1). This 21-d phase will be referred to as Period 2 throughout the remainder of this paper. Feed intake and BW were measured in 7 d intervals within Period 1 and Period 2 to determine average daily gain (ADG), average daily feed intake (ADFI), average daily energy intake (ADEI), feed efficiency (G:F), and energy efficiency (G:E) on a per pen basis.

Blood collection and analyses

Forty sentinel pigs were randomly chosen prior to transport and balanced by *in utero* treatment, diet treatment, and sex. Blood samples were collected (3 mL; serum; BD Diagnostics, Franklin Lakes, NJ) via jugular venipuncture immediately prior to transport (**Pre-T**) and immediately post-transport (**Post-T**). Following transport, the 40 sentinel pigs were distributed evenly (1 pig per pen) and then blood samples were collected at 0800 h on d 2, 7, 14, 28, and 35 of the nursery period. Serum was collected by centrifugation at 4°C and 1900 x g for 15 min, aliquoted, and stored at -80°C. Serum cortisol concentrations were analyzed using a commercially available radioimmunoassay (RIA) kit [minimum detectable level: 0.5 ng/mL; Cortisol RIA (CT), IBL International, Hamburg, Germany]. A commercially available ELISA kit (minimum detectable level: 0.05 ng/mL; Mercodia Porcine Insulin ELISA; Mercodia AB; Uppsala, Sweden) was used to determine insulin concentrations according to the manufacturer's instructions. Commercially available kits were used to determine serum non-esterified fatty acid (NEFA; minimum detectable level: 0.01 mEq/L; Autokit NEFA, Wako Chemicals USA, Richmond, VA) and glucose (minimum detectable level: 0.0 mg/dL; Autokit glucose, Wako chemicals USA, Richmond, VA) concentrations. The intra-assay CVs were 14.5, 10.4, 9.2, and 3.9, for cortisol, insulin, NEFA, and glucose, respectively, and the inter-assay CVs were 16.5, 11.8, 18.3, and 9.3, for cortisol, insulin, NEFA, and glucose, respectively.

Animal Behavior

Piglets were video-recorded immediately following weaning and transport for 14 d upon placement into their assigned pens using ceiling mounted cameras (Panasonic WV-CP254H, Matsushita Electric Industrial Co. Ltd., Osaka, Japan) attached to a digital video recorder system (GeoVision VMS Software; GeoVision Inc., Tapei, Taiwan). Video was recorded during the light and dark periods (12 h: 12 h). Video files were later analyzed using Observer XT 14 behavioral analysis software (Noldus Information Technology B.V., Wageningen, The

Netherlands) by three trained individuals that were blind to the treatments and maintained an agreement of 90% or greater. Individual behaviors were determined using an instantaneous scan sampling technique in 10 min intervals on d 3, 5, 8, 10, and 13 postweaning for 3 periods each day (0800 to 1000 h, 1100 to 1300 h, and 1400 to 1600 h) to encompass periods of greater activity. Behavioral analysis days were selected so that they did not fall on days in which pigs were being handled or blood samples were being taken. Specific behaviors were quantified individually and included posture (lying, standing, other, non-observable), consumption (feeding, drinking, other, non-observable), and huddling (Table 2).

Statistics

All data were analyzed using the PROC GLIMMIX procedure in SAS 9.4 (Cary, NC). The assumptions of normality of error, homogeneity of variance, and linearity were confirmed post-hoc. Sex was included in all IUHS and IUTN piglet analyses but only remained for those analyses where differences were detected. For all repeated analyses, covariance structure was selected based on goodness of fit criterion with day or week as the repeated effect as needed. A statistical significance between comparisons was defined when $P \leq 0.05$ and a tendency was defined as $0.05 < P \leq 0.10$.

Gestating gilt data

For gestating gilt thermoregulatory data, daily averages were calculated for all measures and then gestating environment (TN, HS), day (0 to 59), and interactions were used as fixed effects. Day was included as a repeated effect for all thermoregulatory analyses and covariance structure was selected based on goodness of fit criterion. Thermoregulatory data differences by day will only be presented or discussed when interacting with gestating environment treatment as only gestating environment treatment effects were of interest in the present study and it was expected that thermoregulatory differences would occur by day due to circadian rhythm and/or advancing gestation. For gilt litter performance, litter averages were calculated for all measures and then gestation environment (TN, HS) was used as a fixed effect.

Transport analyses

For analyses of blood parameter data during the transport phase, *in utero* treatment (IUHS, IUTN), collection time (Pre-T, Post-T), and their interactions were used as fixed effects. Litter was included as a random effect.

Growth performance data

Post-T BW was used as a covariate and block was included as a random effect for all post-weaning and transport growth performance measures. *In utero* treatment (IUTN, IUHS), diet treatment (C, ND), week (1, 2, 3, 4, 5), and their interactions were included as fixed effects for the ADG, ADFI, ADEI, G:F, and G:E analyses within Periods 1 and 2. However, week data will only be presented or discussed when interacting with *in utero* or dietary treatment as only *in utero* and dietary treatment effects were of interest in the present study and it was expected that performance differences would occur as pigs advanced in age.

Blood parameter data

In utero treatment (IUTN, IUHS), diet treatment (C, ND), day (2, 7, 14, 28, 35), and their interactions were included as fixed effects for all blood parameter data within Periods 1 and 2. Post-T blood parameter data were used as covariates for all blood parameter analyses.

Behavioral data

In utero treatment (IUTN, IUHS), diet treatment (C, ND), and their interactions were included as fixed effects for the behavioral analyses. Block was included as a random effect for all behavioral analyses. Drinking behavior data were square root transformed to meet assumptions of normality of error, homogeneity of variance, and linearity; however, back-transformed LSmeans and standard errors are presented for clarity.

Results:

Pregnant gilt thermoregulation and litter performance

Heat-stressed pregnant gilts had increased T_R ($P < 0.01$; 0.35°C) compared to TN pregnant gilts, regardless of gestation day (Table 3). Rectal temperature was greater ($P < 0.01$) on d 6 to 10, d 14 to 18, and d 22 to 58 of gestation for HS compared to TN pregnant gilts (Fig. 1A). Respiration rate was increased overall ($P < 0.01$; 25 bpm) in HS compared to TN pregnant gilts (Table 3). An increase ($P < 0.01$) in RR was detected on d 6 to 58 of gestation for HS compared to TN pregnant gilts (Fig. 1B). Ear skin temperature was increased overall ($P < 0.01$; 10.04°C) in HS compared to TN pregnant gilts (Table 3). Heat-stressed pregnant gilts had an increase ($P < 0.01$) in T_{ear} on d 6 to 58 when compared to TN pregnant gilts (Fig. 1C). Shoulder skin temperature was greater overall for HS ($P < 0.01$; 6.10°C) compared to TN pregnant gilts (Table 3). An increase in T_{shoulder} was observed ($P < 0.01$) from d 6 to 58 of gestation in HS compared to TN pregnant gilts (Fig. 1D). No other pregnant gilt thermoregulation differences ($P > 0.05$) related to gestation environment were observed (Table 3; Fig. 1).

Heat-stressed pregnant gilts had an increase in liveborn piglets ($P = 0.03$; 2.25 pigs) compared to TN pregnant gilts (Table 3). Stillborn piglets tended to be reduced ($P = 0.09$; 1.03 pigs) in HS compared to TN pregnant gilts (Table 3). Pregnant gilts exposed to HS had a greater number of weaned piglets ($P = 0.03$; 2.61 pigs) when compared to TN pregnant gilts (Table 3). No other litter performance differences ($P > 0.05$) were detected (Table 3).

Growth Performance

Period 1

A decrease in ADG was detected overall ($P = 0.03$; 10.9%) in IUHS versus IUTN pigs (Table 4). Regardless of *in utero* treatment, ADFI was reduced ($P = 0.02$; 8.9%) in ND compared to C fed pigs (Table 4). Feed efficiency tended to be increased ($P = 0.10$; 3.5%) for ND compared to C pigs, regardless of *in utero* treatment (Table 4). No other growth performance differences ($P > 0.05$) were detected (Table 4).

Period 2

Overall, ADFI was increased ($P = 0.05$; 4.4%) in ND compared to C fed pigs (Table 4). No other growth performance differences were detected ($P > 0.05$; Table 4).

Blood Parameters

Transport phase

Serum cortisol concentrations were decreased overall ($P = 0.03$; 25.8%) in IUHS compared to IUTN pigs (Table 5). Post-T cortisol concentrations were greater ($P < 0.01$; 48.3%) than Pre-T levels, regardless of *in utero* treatment (Table 6). Circulating glucose levels were increased overall ($P = 0.04$) in barrows (131.1 ± 3.3 mg/dL) compared to gilts (121.83 ± 3.1 mg/dL; data not presented). Overall, Post-T NEFA concentrations were increased ($P = 0.03$; 26.9%) when compared to Pre-T concentrations, regardless of *in utero* treatment (Table 6). No other blood parameter differences were detected ($P > 0.05$) with any comparison (Table 5, 6; Fig. 1, 2).

Period 1

Overall, serum cortisol concentrations were decreased ($P < 0.01$) on d 7 (27.60 ± 1.96 ng/mL) and d 14 (28.07 ± 2.95 ng/mL) compared to d 2 (45.72 ± 4.90 ng/mL; Fig. 2). Circulating insulin levels were greater on d 7 (0.216 ± 0.030 ng/mL) and d 14 (0.267 ± 0.030 ng/mL) compared to d 2 (0.079 ± 0.010 ng/mL), regardless of *in utero* or dietary treatment (Fig. 3A). Insulin was increased overall ($P = 0.02$; 51.0%) in ND versus C pigs (Table 5). Blood glucose was increased on d 7 (119.4 ± 3.0 mg/dL) and d 14 (119.1 ± 2.3 mg/dL) compared to d 2 (97.3 ± 3.2 mg/dL), regardless of *in utero* or dietary treatment (Fig 3B). A decrease ($P = 0.01$) in blood glucose was observed for IUHS (90.6 ± 4.4 mg/dL) compared to IUTN (104.1 ± 4.6 mg/dL) pigs on d 2, regardless of dietary treatment (Fig. 3B). Insulin:glucose was increased on d 7 (0.17 ± 0.02) and d 14 (0.22 ± 0.02) compared to d 2 (0.08 ± 0.02), regardless of *in utero* or dietary treatment (Fig 3C). An overall increase in I:G was detected ($P = 0.03$; 38.5%) in ND compared to C pigs (Table 5). Non-esterified fatty acids were decreased ($P < 0.01$) on d 7 (100.8 ± 9.5 mEq/L) and d 14 (106.0 ± 13.3 mEq/L) compared to d 2 (810.3 ± 61.1 mEq/L), regardless of *in utero* and dietary treatment (Fig. 3D). No other blood parameter differences were detected ($P > 0.05$) with any comparison (Table 5; Fig. 2, 3).

Period 2

Overall, circulating insulin tended ($P = 0.07$) to be reduced (21.7%) in IUHS compared to IUTN pigs (Table 5). No other blood parameter differences were detected ($P > 0.05$) with any comparison (Table 5; Fig. 2, 3).

Behavior

Overall, lying behavior was reduced ($P = 0.03$; 4.4%) in ND versus C pigs (Table 7). Lying behavior was greater ($P < 0.01$) on d 3 ($69.2 \pm 1.8\%$) and d 5 ($68.4 \pm 1.4\%$) when compared to d 8 ($63.1 \pm 1.5\%$), d 11 ($64.9 \pm 1.3\%$), and d 13 ($63.8 \pm 1.2\%$), regardless of *in utero* or dietary treatment (Fig. 4A). Standing behavior was increased overall ($P = 0.04$; 4.0%) in ND compared to C pigs (Table 7). An overall reduction in standing behavior was detected on d 3 ($22.7 \pm 1.4\%$) and d 5 ($27.5 \pm 1.4\%$) versus d 8 ($34.4 \pm 1.4\%$), d 11 ($33.0 \pm 1.4\%$), and d 13 ($34.4 \pm 1.4\%$), regardless of *in utero* or dietary treatment (Fig. 4B). No other behavioral differences were detected ($P > 0.05$) with any comparison (Table 7, Fig. 4).

Discussion:

Heat stress during gestation negatively impacts the dam (Omvedt et al., 1971) and influences the long-term postnatal development of her offspring (Johnson et al., 2015; Chapel et al., 2017). Because developing fetuses lack the capacity to thermoregulate *in utero* (Schroder and Power, 1994, 1997; Ziskin and Morrissey, 2011), they are completely dependent on the dam's body temperature and placental blood flow to dissipate excess heat through thermal gradients. Unfortunately, increasing maternal body temperature compromises this gradient thereby putting fetal development at risk and increasing embryonic mortality (Tompkins et al., 1967; Sakatani et al., 2004). In the present study, HS pregnant gilts had an increase in all thermoregulatory measures (e.g., T_R , RR, T_{ear} , $T_{shoulder}$) when compared to TN pregnant gilts. These results were expected as previous research by our group (Lucy et al., 2012b; Chapel et al., 2017) and others (Omvedt et al., 1971) have observed increased body temperature measures in pregnant gilts exposed to similar elevated T_A ranges, and this likely indicates that HS pregnant gilts (and subsequently their developing offspring) were suffering from HS. However, despite the fact that HS pregnant gilts were suffering from HS, the number of live born piglets and the number of weaned piglets per litter were increased for HS compared to TN pregnant gilts. These results were surprising considering the previously described reductions in live born and weaned piglets for HS pregnant sows (Omvedt et al., 1971; Wildt et al., 1975). However, these data may be a result of HS timing because embryonic losses influencing litter size and pigs weaned are thought to occur in the first week of fetal development (Tompkins et al., 1967; Sakatani et al., 2004) and HS-exposure in the present study did not occur until d 6 post-artificial insemination. This hypothesis may be further confirmed by the fact that research in gestating sows that are exposed to HS after the first week post-breeding have no litter size or weaning weight differences (Williams et al., 2013), and it is likely that the improvements in live born and number of piglets weaned for HS pregnant gilts in the present study are not a direct result of the environmental treatment. While HS pregnant gilts had increased live born and pigs weaned per litter, no birth weight or gestation length differences were observed between HS and TN pregnant gilts. These results are contradictory to previous studies that reported reductions in birth weight and gestation length in pigs (Lucy et al., 2012) and dairy cattle (Tao et al., 2012); however, these discrepancies may again be explained by differences in HS timing. This is because the majority of fetal BW is gained during the last half of gestation (via exponential growth) and late-gestation HS is linked to shorter gestation lengths in pigs (Lucy et al., 2012) that would likely reduce the amount of BW gain over the final days of gestation. Therefore, because HS was applied only during the first half of gestation in the present study and all pregnant gilts were maintained under TN conditions for the second half of gestation, this may explain the lack of gestation length and birth weight differences between HS and TN pregnant gilts. Regardless of the lack of initial litter performance differences, the influence of IUHS on postnatal development in pigs is well-described (Johnson and Baumgard, 2019) and likely played a role in the phenotypes observed for IUHS offspring in the present study.

In utero heat stress increases FHP in pigs during the growing phase of postnatal development (Chapel et al., 2017). However, feed intake does not increase to compensate for the greater energetic demands (Johnson et al., 2015), and this may put pigs at an energetic disadvantage and increase stress, especially during times of low energy intake such as weaning and transport (Byrd et al., 2019). To combat this, IUHS pigs were fed a ND diet formulated to

increase net energy intake following weaning and transport and improve growth performance. Despite this however, it was demonstrated that IUHS pigs had an overall reduction in ADG compared to IUTN pigs following weaning and transport. The decrease in growth performance for IUHS compared to IUTN pigs may have been related to the aforementioned increase in FHP (Chapel et al., 2017) that was not rescued by increased energy intake because pigs fed the ND diet had reduced ADFI and similar ADEI when compared to the C diet fed pigs. Interestingly, while not statistically significant, a numerical reduction in G:F was observed in IUHS pigs that may also point towards an increase in FHP (Patience, 2012). Reasons why pigs fed the ND diet adjusted their ADFI to match the ADEI of the C diet fed pigs may be related to the fact that energy density is the first factor affecting ADFI in pigs (Henry et al., 1985), and ADFI decreases as energy content of the diet increases (Beaulieu et al., 2009). However, the decrease in ADFI in the present study was unexpected because the aforementioned ADFI response has primarily been observed in pigs greater than 20 kg (Black et al., 1986), and pigs in the present study never reached this weight. Therefore, while more research should be conducted to confirm this response in smaller pigs, results from this study suggest that increasing the net energy of the diet may not be an effective strategy to improve growth performance in IUHS pigs following weaning and transport.

Blood glucose levels are reduced during times of fasting or energy intake that is not sufficient to meet a pig's needs (i.e., following weaning and transport; Sutherland et al., 2010). As a result, insulin levels are reduced resulting in glycogenolysis to maintain blood glucose levels and lipolysis to mobilize adipose tissue to meet the metabolic requirements of the pig (Inoue et al., 2005). In the present study, blood glucose was reduced overall on d 2 compared to d 7 and 14 following weaning and transport, and this corresponds with a time period in which feed intake is generally low in pigs (Johnson and Lay, 2017). In addition to the overall reduction in blood glucose levels, a decrease in blood glucose was observed on d 2 for IUHS compared to IUTN pigs. Although ADEI was not measured on d 2, no overall ADEI differences were observed between IUHS and IUTN pigs during the time frame in which blood parameters were measured. Therefore, this response may be related to the aforementioned increase in energy requirements for IUHS pigs (Chapel et al., 2017), because glucose demand is increased in animals with higher metabolic rates (Eissen, 2000). Although blood glucose levels were reduced for IUHS pigs on d 2, no corresponding decrease in insulin occurred, and this may explain the similar circulating NEFA concentrations for IUHS compared to IUTN pigs. This response is similar to a previous report in weaned and transported IUHS pigs (Byrd et al., 2019) and may be a result of hyperinsulinemia in IUHS pigs as previously described (Boddicker et al., 2014). The hyperinsulinemia response likely places IUHS pigs at a metabolic disadvantage during times of low energy intake, and this metabolic inflexibility that prevents energy mobilization may increase the stress response of IUHS pigs following weaning and transport as previously described (Byrd et al., 2019).

The stress of weaning and transport may be compounded in IUHS compared to IUTN pigs. Previous research demonstrates that IUHS pigs have increased circulating cortisol levels when exposed to novel stressors (Chapel et al., 2017), immediately following birth (Machado-Neto et al., 1987), and have increased salivary cortisol following mixing stress (Merlot et al., 2019), and these data suggest that IUHS pigs may be more stress sensitive. However, despite the fact that circulating cortisol levels were increased Post-T compared to Pre-T for all pigs (likely indicating a greater stress response; Bradshaw et al., 1996), this response was blunted in IUHS compared to IUTN pigs. Furthermore, no *in utero* treatment-related cortisol differences were

observed following the transport phase. Decreased blood cortisol is generally associated with a reduced stress response (Marchant-Forde et al., 2012), and while these data contradict our hypothesis, they confirm a previous report in which circulating cortisol was reduced in IUHS compared to IUTN pigs that were weaned and transported (Byrd et al., 2019). While reasons for this response and the inconsistency with other reports in IUHS pigs (Machado-Neto et al., 1987; Chapel et al., 2017; Merlot et al., 2019) require further investigation, it was previously speculated that IUHS may down regulate the HPA-axis feedback set point resulting in decreased postnatal cortisol release in response to a stressor (Byrd et al., 2019). However, a downregulated HPA-axis feedback setpoint does not explain the inconsistencies in the cortisol response of IUHS pigs when comparing the acute stress response [i.e., greater cortisol following birth (Machado-Neto et al., 1987), novel stressors (Chapel et al., 2017), and mixing stress (Merlot et al., 2019)] and the chronic stress response [i.e., reduced cortisol following weaning and 7 h 5 min transport (Byrd et al., 2019) or 11 h 40 min transport in the present study]. While it is possible that the decreased Post-T cortisol response for IUHS compared to IUTN pigs indicates a reduced stress response, more research should be performed to evaluate the underlying mechanism(s) for the inconsistencies in the cortisol response of IUHS pigs as it relates to the acute versus chronic response.

The physiological stress response is inextricably linked to the behavioral response of pigs. Therefore, changes in animal physiology may result in behavioral alterations under stressful conditions. Following weaning and transport, pigs increase their lying behavior and reduce their activity (Lewis, 2008), and this response was observed in the present study regardless of *in utero* or dietary treatment. In addition, greater aggression and reduced feeding and drinking behaviors are a common occurrence (Melotti et al., 2011). However, IUHS may alter the incidence of these behaviors and IUHS pigs display increased drinking behavior (polydipsia) and a trend for greater aggression in the 7-d following weaning and transport, and this has been attributed to a greater stress response (Byrd et al., 2019). In addition, studies have shown a decrease in activity and an increase in lying behavior for IUHS pigs in the first 48 h post-weaning (Merlot et al., 2019). Despite the aforementioned behavioral alterations observed for newly weaned and/or transported IUHS pigs, no *in utero* treatment-related behavioral differences were observed in the present study, and this may be attributed to study design differences. In the previous studies (Byrd et al., 2019; Merlot et al., 2019), behavioral analyses were conducted in the first 48 h post-weaning, while in the present study, behavioral data collection days were selected so that they did not fall on days in which pigs were being disturbed by handling or blood collection, which excluded the first 48 h post-weaning and transport. Therefore, because the majority of behavioral (Byrd et al., 2019; Merlot et al., 2019) and physiological (Orgeur et al., 2001; Lewis et al., 2005) changes in pigs appear to occur in the first 48 h post-weaning, it is possible that any *in utero* treatment differences that may have been present were missed in the analysis. Therefore, future behavioral research in IUHS pigs should focus on the first 2 days immediately following weaning and transport, as any behavioral differences appear to dissipate by day 3.

Although no *in utero* treatment-related behavioral differences were detected, ND fed pigs displayed decreased lying behavior and increased standing behavior relative to C fed pigs. Although variation related to housing, genetics, and behavioral data collection time intervals exists among studies (Lewis, 2008), in general, increased lying and decreased standing behaviors have previously been associated with increased sickness level in response to an immune challenge in pigs (Johnson and Von Borell, 1994; Escobar et al., 2007). Therefore, it is possible that the decreased lying and increased standing behavior indicates that ND fed pigs were at a

higher health status when compared to C fed pigs. Alternatively, these behavioral changes may be associated with decreased ADFI for ND compared to C fed pigs because previous studies in pigs have correlated reduced feed intake with increased standing behavior (Appleby and Lawrence, 1987). Although these diet-treatment-related behavioral changes are interesting, they do not appear to be associated with any growth performance, stress response, or metabolic differences and so the relative importance of these changes to the health and welfare of the pigs in the present study is currently unknown.

Conclusions:

The process of weaning and transport is one of the most stressful periods in a pig's life. Unfortunately, the effects of IUHS on postnatal FHP may compound these negative effects resulting in reduced growth performance, increased stress, and decreased welfare when compared to IUTN counterparts. Therefore, it was hypothesized that providing IUHS pigs with ND diets following weaning and transport would improve their postnatal growth performance and stress response. It was determined that providing IUHS pigs with ND diets did not improve their growth performance and that ADG was reduced overall when compared to IUTN pigs. The decrease in ADG for IUHS pigs was likely due to the fact that ND diet fed pigs had reduced ADFI and similar ADEI as C diet fed pigs. In addition to reduced growth performance, IUHS pigs had metabolic alterations precluding them from mobilizing energy reserves following weaning and transport when compared to IUTN pigs. These data indicate that IUHS is detrimental to future growth performance following weaning and transport and that this negative impact is not rescued by the provision of ND diets.

Acknowledgements:

The authors would like to acknowledge the University of Missouri and Purdue University Swine Farm staff and students as well as the USDA-ARS Livestock Behavior Research Unit employees for their help with data collection, animal care, and lab analyses.

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Table 1: Nursery phase diet compositions

Item	Phase 1 ¹		Phase 2 ²	
	C ³	ND ⁴	C	ND
<i>Ingredients, % as fed</i>				
Corn	31	25.68	37.775	18.475
Soybean Meal, 48% CP	13.94	18	18	13.94
Soybean Isolate	-	4.1	-	4.37
Soybean oil	5	12.45	5	12.57
Monocal. Phosphate	0.38	0.84	0.49	0.77
Limestone	0.8	0.84	0.74	0.9
Salt	0.25	0.25	0.25	0.25
Lysine -HCl	0.07	0.2	0.2	0.07
DL-Methionine	0.22	0.29	0.225	0.29
L-Threonine	0.04	0.12	0.09	0.065
L-Tryptophan	0.01	0.015	0.015	0.01
Swine Vit. Premix	0.25	0.25	0.25	0.25
Swine TM Premix	0.125	0.125	0.125	0.125
Selenium premix	0.05	0.05	0.05	0.05
Dried Whey	25	25	25	25
Lactose	5	-	-	5
Sel. Men. Fish Meal	5	4	4	5
Plasma Protein	6.5	2.5	2.5	6.5
SD Blood Meal	1.5	1.5	1.5	1.5
Soy Concentrate	4	3	3	4
Zinc Oxide	0.375	0.35	0.35	0.375
Carbadox 10	0.25	0.25	0.25	0.25
Phytase, 600 PU/g	0.1	0.1	0.1	0.1
Clarify larvacide	0.14	0.09	0.09	0.14
<i>Calculated chemical composition</i>				
NE, kcal/lb	1252.9	1389.4	1240.6	1403.2
NE, kcal/kg	2762.2	3063.1	2735.1	3093.5
Crude Protein, %	24.59	25.43	22.85	27.37
SID Lys, %	1.55	1.62	1.45	1.74
SID Lys:NE	5.61	5.3	5.3	5.61
Ca, %	0.9	0.95	0.85	1.01
P avail., %	0.6	0.61	0.55	0.67
<i>Analyzed chemical composition</i>				
GE, kcal/kg	4524.08	4058.37	4552.31	4097.05
CP, %	26.89	24.60	23.46	21.34
Total Lys, %	1.74	1.65	1.73	1.99

Ca, %	0.98	0.89	1.11	0.97
P, %	0.67	0.58	0.67	0.57

Table 1: *Continued*

¹Fed days 0 to 7 postweaning and transport

²Fed days 7 to 14 postweaning and transport

³Pigs provided C diet

⁴Pigs provided ND diet

Table 2: Ethogram used for behavior analyses.

Behavior	Description
<i>Posture</i>	
Standing	Standing on 4 legs.
Lying	Pig is sternal or lateral on the ground. Feet do not support weight of piglet.
Other	Piglet visible but is not standing or lying. i.e. kneeling or sitting.
Non-observable	Piglet is not visible.
<i>Consumption</i>	
Feeding	Head positioned inside of the feeder with oral movement.
Drinking	Drinking water from the nipple-drinker.
Other	Piglet visible but not eating or drinking.
Non-observable	Piglet is not visible.
<i>Huddling</i>	
Huddling	In contact with one or more pen mates.
Not Huddling	Not in contact with one or more pen mates.
Non-observable	Piglet is not visible.

Table 3: Effects of gestation environment on pregnant gilt thermoregulation and litter performance.

Parameter	Gestation Environment		SEM	P-value
	TN	HS		E ¹
<i>Thermoregulation</i>				
T _R , °C	38.08	38.43	0.04	<0.01
RR, bpm	17	42	1	<0.01
T _{ear} , °C	23.95	33.99	0.31	<0.01
T _{shoulder} , °C	29.09	35.19	0.16	<0.01
<i>Litter performance</i>				
Anogenital distance, cm	6.28	6.95	0.62	0.46
Birth weight, kg	1.25	1.37	0.12	0.46
Gestation length, d	113.67	113.92	0.37	0.64
Live born, piglets/litter	10.67	12.92	0.69	0.03
Still born, piglets/litter	1.11	0.08	0.40	0.09
Weaned, piglets/litter	9.56	12.17	0.79	0.03
Week 1 BW, kg	2.52	2.51	0.13	0.96
Week 2 BW, kg	4.19	4.21	0.20	0.94
Weaning BW, kg	4.83	4.83	0.22	0.99

¹Environmental treatment

Differences at $P \leq 0.05$.

Tendencies at $0.05 < P \leq 0.10$

Table 4: Effects of IUHS on postnatal growth performance parameters in pigs fed a C or ND diet following weaning and transport.

Parameter	<i>In utero</i> treatment		Diet treatment		SEM	<i>P</i> -value	
	IUTN	IUHS	C	ND		IU ¹	D ²
<i>Period 1</i>							
ADG, g/d	221	197	212	206	8	0.03	0.48
ADFI, g/d	265	253	271	247	9	0.22	0.02
ADEI, kcal/d	1139	1091	1095	1135	46	0.24	0.66
G:F, kg/kg	0.86	0.83	0.83	0.86	0.02	0.13	0.10
G:E, kg/kcal	0.20	0.20	0.19	0.21	0.01	0.69	0.38
d 14 BW, kg	7.84	7.69	7.72	7.81	0.32	0.42	0.63
<i>Period 2</i>							
ADG, g/d	504	491	490	506	10	0.39	0.31
ADFI, g/d	841	820	812	849	9	0.27	0.05
ADEI, kcal/d	3341	3317	3334	3324	79	0.73	0.93
G:F, kg/kg	0.51	0.49	0.49	0.51	0.01	0.33	0.34
G:E, kg/kcal	0.15	0.15	0.15	0.15	<0.01	0.70	0.94
d 35 BW, kg	18.36	18.41	18.29	18.48	0.57	0.86	0.49

¹*In utero* treatment²Dietary treatmentDifferences at $P \leq 0.05$.Tendencies at $0.05 < P \leq 0.10$

Table 5: Effects of IUHS on blood parameters in pigs fed a C or ND diet following weaning and transport.

Parameter	<i>In utero</i> treatment		Diet treatment		SEM	<i>P</i> -value	
	IUTN	IUHS	C	ND		IU ¹	D ²
<i>Transport Phase</i>							
Cortisol, ng/mL	48.69	36.15	-	-	4.06	0.03	-
Insulin, ng/mL	0.157	0.164	-	-	0.090	0.85	-
Glucose, mg/dL	127.7	124.3	-	-	3.1	0.45	-
I:G	0.13	0.12	-	-	0.02	0.78	-
NEFA, mEq/L	343.3	327.9	-	-	24.7	0.66	-
<i>Period 1</i>							
Cortisol, ng/mL	34.65	32.92	34.44	33.12	2.44	0.63	0.71
Insulin, ng/mL	0.192	0.182	0.149	0.225	0.020	0.76	0.02
Glucose, mg/dL	111.3	114.2	113.3	112.2	2.1	0.34	0.73
I:G	0.16	0.15	0.13	0.18	0.02	0.69	0.03
NEFA, mEq/L	347.9	330.3	322.5	355.6	27.0	0.65	0.39
<i>Period 2</i>							
Cortisol, ng/mL	35.87	41.93	38.44	39.36	3.67	0.29	0.86
Insulin, ng/mL	0.437	0.342	0.359	0.420	0.040	0.07	0.24
Glucose, mg/dL	122.5	123.2	122.4	123.3	2.3	0.83	0.79
I:G	0.34	0.31	0.31	0.34	0.03	0.48	0.63
NEFA, mEq/L	57.8	49.3	51.3	55.7	5.9	0.28	0.58

¹*In utero* treatment²Dietary treatmentDifferences at $P \leq 0.05$.Tendencies at $0.05 < P \leq 0.10$

Table 6: Effects of transport on piglet blood parameters

Parameter	Collection Time			<i>P</i> -value
	Pre-T	Post-T	SEM	C
Cortisol, ng/mL	28.93	55.91	4.06	<0.01
Insulin, ng/mL	0.159	0.162	0.023	0.91
Glucose, mg/dL	128.29	124.64	3.04	0.37
I:G	0.12	0.13	0.02	0.35
NEFA, mEq/L	295.8	375.4	24.7	0.03

Differences at $P \leq 0.05$.

Tendencies at $0.05 < P \leq 0.10$.

Table 7: Effects of IUHS on the behavior of pigs fed a C or ND diet following weaning and transport.

Parameter	In utero treatment		Diet treatment		SEM	P-value	
	IUTN	IUHS	C	ND		IU ¹	D ²
<i>Posture</i>							
Lying, %	65.9	65.9	68.1	63.7	<0.1	0.99	0.03
Standing, %	30.1	30.7	28.4	32.4	<0.1	0.73	0.04
Other, %	4.6	3.6	4.4	4.1	2.3	0.26	0.62
Non-observable, %	2.9	2.3	2.6	2.6	1.0	0.98	0.38
<i>Consumption</i>							
Feeding, %	9.3	10.3	8.9	10.8	2.8	0.45	0.15
Drinking, %	4.6	4.0	4.1	4.6	<0.1	0.45	0.52
Other, %	82.1	82.3	83.2	81.3	0.1	0.86	0.12
Non-observable, %	2.9	2.3	4.4	4.1	2.3	0.26	0.62
Huddling, %	96.2	49.6	81.2	64.6	26.2	0.22	0.66

¹*In utero* treatment²Dietary treatmentDifferences at $P \leq 0.05$.

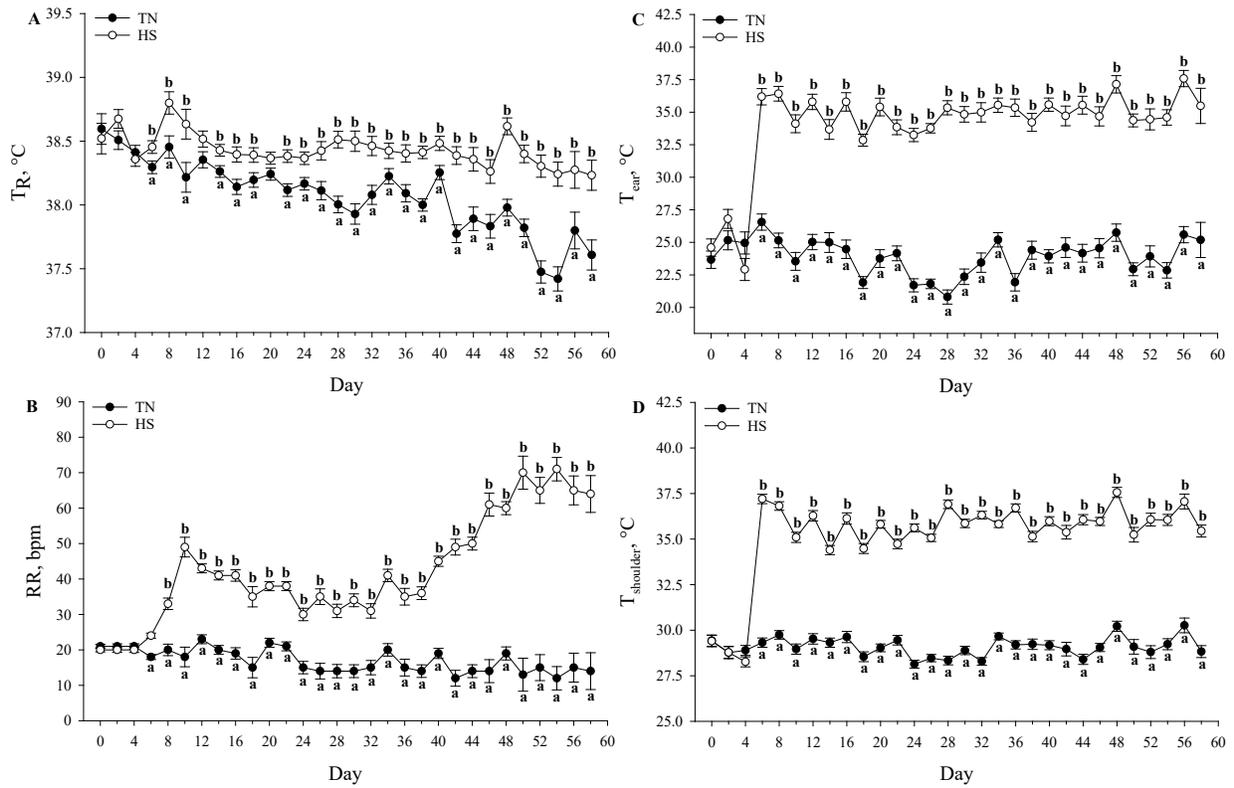


Figure 1: Effects of gestation environment on (A) T_R , (B) RR, (C) T_{ear} , and (D) $T_{shoulder}$ by day post-artificial insemination. Error bars indicate ± 1 SEM. ^{a,b}Letters indicate differences ($P < 0.05$) comparing gestation environment by day post-artificial insemination.

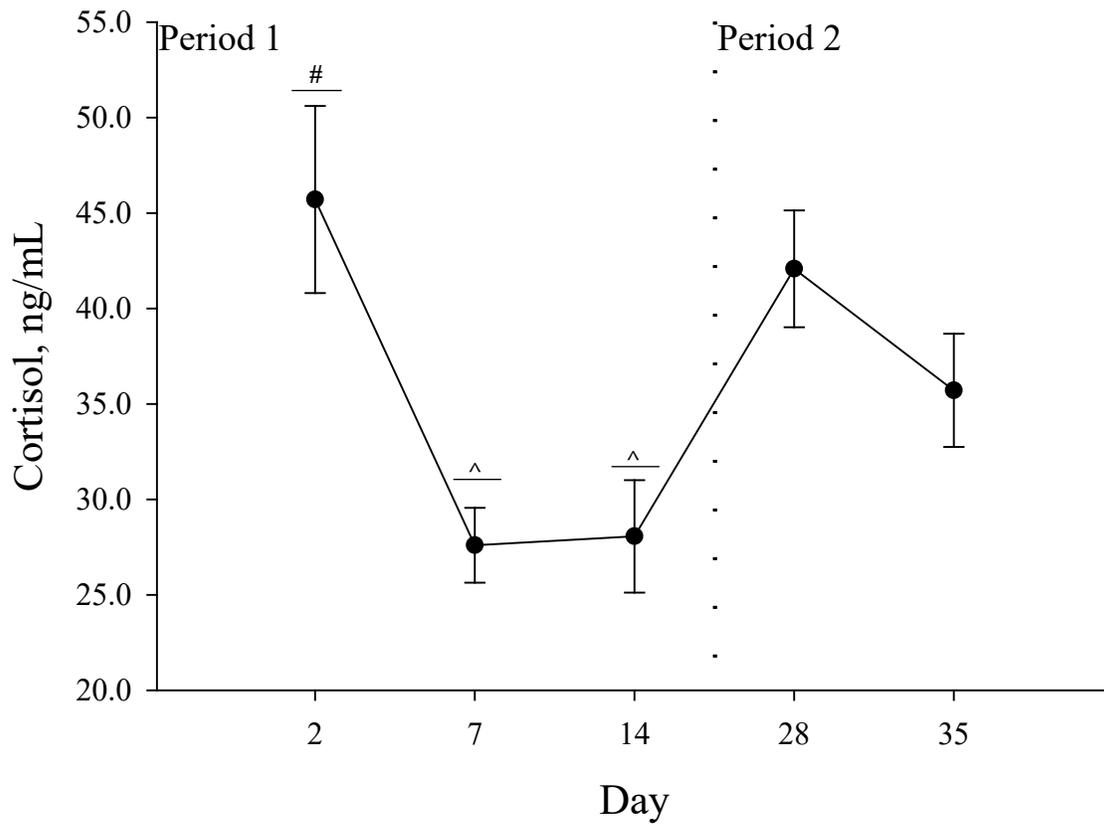


Figure 2: Cortisol by day post-weaning and transport during Period 1 (d 0 to 14 post-weaning and transport) and Period 2 (d 15 to 35 post-weaning and transport). Error bars indicate ± 1 SEM. #, ^ Symbols indicate differences ($P < 0.05$) comparing blood parameter by day post-weaning and transport.

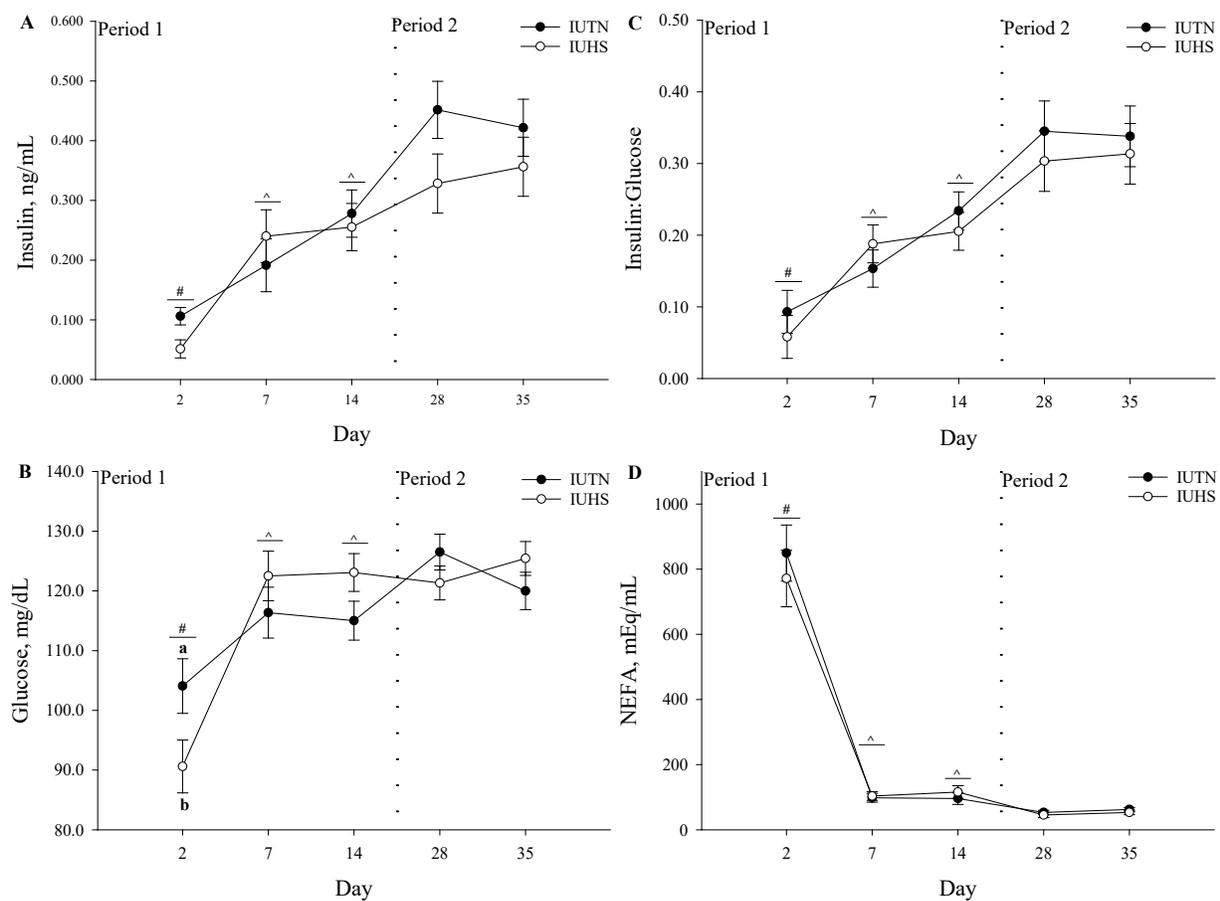


Figure 3: Effects of *in utero* treatment on postnatal circulating (A) insulin, (B) glucose, (C) I:G, and (D) NEFA in pigs following weaning and transport. Error bars indicate ± 1 SEM. ^{a,b}Letters indicate differences ($P < 0.05$) comparing *in utero* treatment by day post-weaning and transport. #, ^ Symbols indicate differences ($P < 0.05$) by day post-weaning and transport.

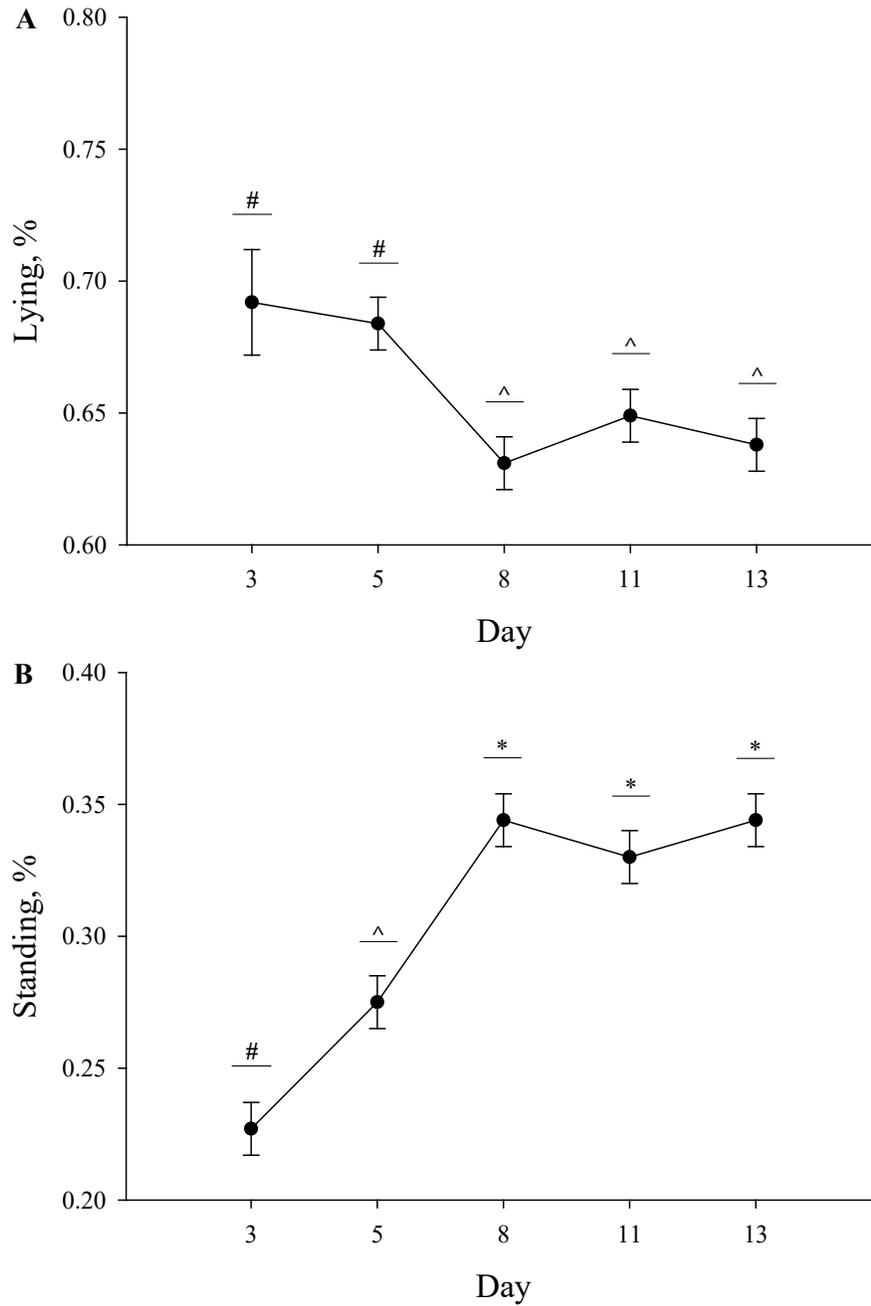


Figure 4: The effects of day post-weaning and transport on (A) lying behavior and (B) standing behavior during Period 1 (d 0 to 14 post weaning and transport). Error bars indicate ± 1 SEM. #, ^ Symbols indicate differences ($P < 0.05$) by day post-weaning and transport.