Effects of heat stress and insulin sensitizers on pig adipose tissue

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Abstract: Heat stress (HS) negatively impacts several swine production variables, including carcass fat quality and quantity. Pigs reared in HS have more adipose tissue than energetically predicted, explainable, in part, by HS-induced hyperinsulinemia. Study objectives were to evaluate insulin's role in altering fat characteristics during HS via feeding insulin-sensitizing compounds. Forty crossbred barrows (113 ± 9 kg BW) were randomly assigned to one of five environment by diet treatments: 1) thermoneutral (TN) fed ad libitum (TNAL), 2) TN and pair-fed (TNPF), 3) HS fed ad libitum (HSAL), 4) HS fed ad libitum with sterculic oil (SO) supplementation (HSSO; 13 g/d), and 5) HS fed ad libitum with dietary chromium (Cr) supplementation (HSCr; 0.5 mg/d; Kemin Industries, Des Moines, IA). The study consisted of three experimental periods (P). During P0 (2 d), all pigs were exposed to TN conditions (23 ± 3 °C, $68 \pm 10\%$ RH) and fed ad libitum. During P1 (7 d), all pigs received their respective dietary supplements, were maintained in TN conditions, and fed ad libitum. During P2 (21 d), HSAL, HSSO, and HSCr pigs were fed ad libitum and exposed to cyclical HS conditions (28 to 33 °C, $58 \pm 10\%$ RH). The TNAL and TNPF pigs remained in TN conditions and were fed

ad libitum or pair-fed to their HSAL counterparts. Rectal temperature (T_R), respiration rate (RR), and skin temperature (T_S) were obtained daily at 0600 and 1800 h. At 1800 h, HS exposed pigs had increased T_R , RR, and T_S relative to TNAL controls (1.13 °C, 48 bpm, and 3.51 °C, respectively; $P < 0.01$). During wk 2 and 3 of P2, HSSO pigs had increased 1800 h T_R relative to HSAL and HSCr (~ 0.40 and ~ 0.42 °C, respectively; $P \leq 0.05$). Heat stress decreased ADFI and ADG compared to TNAL pigs (2.24 vs. 3.28 and 0.63 vs. 1.09 kg/d, respectively; $P < 0.01$) and neither variable was affected by SO or Cr supplementation. Heat stress increased or tended to increase moisture content of abdominal (7.7 vs. 5.9%; $P = 0.07$) and inner s.c. (11.4 vs. 9.8%; $P < 0.05$) adipose depots compared to TNAL controls. Interestingly, TNPF pigs also had increased adipose tissue moisture content and this was most pronounced in the outer s.c. depot (15.0 vs. 12.2%; $P < 0.01$) compared to TNAL pigs. Heat stress had little or no effect on fatty acid composition of abdominal, inner, and outer s.c. adipose tissue depots. In summary, the negative effects of HS on fat quality do not appear to be fatty acid composition related, but may be explained by increased adipose tissue moisture content.

Key words: adipose tissue, fatty acid composition, heat stress, insulin

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INTRODUCTION

Heat stress (HS) impedes efficient pork production by reducing feed intake, altering metabolism, and ultimately compromising the animal's

ability to express its genetic potential for maximum growth (Baumgard and Rhoads, 2013). Paradoxically, animals, including pigs, reared during HS accumulate more carcass fat than their feed intake predicts (Close et al., 1971; Collin et al., 2001; Pearce et al., 2013), likely stemming from HS-induced hyperinsulinemia (Baumgard and Rhoads, 2013). However, less is known about how HS influences adipose fatty acid (FA) characteristics. Animals strategically modify FA saturation in an attempt to maintain proper membrane fluidity in response to differing ambient temperatures, a phenomenon referred to as homeoviscous adaptation (Hazel, 1995). The impact of HS on farm animal FA saturation is relevant because it affects meat processing, product quality, and shelf life (Wood et al., 2008).

Cells regulate FA saturation primarily through stearoyl-CoA desaturase (SCD) for which activity and quantity is regulated by insulin (Dobrzyn et al., 2010). Insulin is also a potent adipogenic signal, a proliferative process that can decrease overall adipocyte size and, consequently, enhance fat pliability (Mendizabal et al., 2004). Therefore, we hypothesized altered carcass fat characteristics could be mediated by HS-induced hyperinsulinemia. Through its interaction with chromodulin, chromium (Cr) potentiates insulin action (Chen et al., 2006; Vincent, 2013) and sterculic oil (SO) also improves insulin sensitivity (Ortinau et al., 2012, 2013), although SO also inhibits SCD activity by binding the enzyme's active site (Corl et al., 2001). To investigate insulin's potential role in the aforementioned parameters, we evaluated the ability of these insulin-sensitizing compounds (Cr and SO) to alter the impact of HS on carcass fat characteristics during a 21-d HS challenge.

MATERIALS AND METHODS

Animals and Experimental Design

All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Forty crossbred barrows (113 ± 9 kg BW) were randomly assigned to one of five diet by environmental treatments: 1) thermoneutral (TN) conditions and ad libitum fed (TNAL; $n = 8$), 2) TN pair-fed (TNPF; $n = 8$), 3) HS conditions and ad libitum fed (HSAL; $n = 8$), 4) HS and ad libitum fed a diet with SO supplementation (HSSO; 13 g/d; $n = 8$), or 5) HS ad libitum fed a diet with Cr supplementation (HSCr; 0.5 mg/d, KemTRACE chromium propionate, Kemin Industries, Des

Moines, IA; $n = 8$). Pigs were housed in individual pens (57×221 cm; 24 pens/room) at the Iowa State University Swine Nutrition Farm research facility (Ames, IA). Each pen was equipped with a stainless steel feeder and a nipple drinker. Water was provided ad libitum during the entire experiment.

All pigs were fed a standard diet consisting mainly of corn and soybean meal formulated to meet or exceed nutrient requirements for energy, amino acids, protein, minerals, and vitamins (NRC, 2012; Table 1). Three dietary supplements were formulated and mixed according to the following specifications: 1) a control supplement consisting of 30 g of a palatable carrier (cookie dough, Do-Biz Foods, LLC, Ames, IA), 2) a homogenized sample of seeds from the *Sterculia foetida* tree with the palatable carrier (13 g of sterculic seeds and 30 g of cookie dough/d), and 3) a Cr supplement with the palatable carrier (0.5 mg Cr and 30 g cookie dough/d). Sterculic seeds were obtained from the Montgomery Botanical Center (Miami, FL), stripped of their seed coat, and minced into < 0.5 cm pieces. The palatable carrier was a strategy to ensure supplement consumption. The SO dose was selected on a metabolic BW basis based on previous rodent reports (Ortinau et al., 2012, 2013). Each supplement was administered *per os* once daily at 0600 h.

This study was divided into three experimental periods (P): P0, P1, and P2. Period 0 (2 d in length) served as an acclimation P in which all pigs were

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

Ingredients	%
Corn	73.77
Soybean meal	9.36
Dried distillers grains	15.00
45-30 vitamin and mineral premix ¹	1.65
L-lysine HCL	0.22
Calculated chemical composition %	
DM	87.3
Crude protein	17.45
Crude fat	3.25
ADF	4.42
NDF	12.06
Ash	3.75

¹0.97% Limestone, 0.37% Salt, 0.18% Dried distillers grains, 0.11% Vitamin and Trace Mineral (Provided 7,279 IU vitamin A, 1,335 IU vitamin D, 39 IU vitamin E, 2 IU vitamin K, 19 mg niacin, 15 mg pantothenic acid, 4 mg riboflavin, 4 mg choline, 0.4 µg folic acid, 24 µg vitamin B12, 1 µg biotin, 214 ppm zinc, 103 ppm manganese, 278 ppm iron, 39 ppm copper, 2 ppm iodine, 0.5 ppm selenium per kg of diet), 0.02% Rono M 10,000.

housed individually in TN conditions (23 ± 3 °C, $68 \pm 10\%$ relative humidity [RH]) with a 12:12 h light–dark cycle and fed ad libitum. During P1 (7 d), pigs received their respective dietary supplements while in TN conditions and fed ad libitum. During P2 (21 d), HSAL, HSSO, and HSCr pigs were fed ad libitum and exposed to cyclical HS conditions with ambient temperatures ranging from 33 °C (0800 to 1800 h; $56 \pm 8\%$ RH) to 28 °C (1800 to 0800 h; $60 \pm 10\%$ RH). The TNAL and TNPF pigs remained in TN conditions and were fed ad libitum or pair-fed to the HSAL counterparts to eliminate the confounding effect of dissimilar feed intake, respectively. Daily feed intake in P1 was averaged for each HSAL pig and used as a baseline; the decrease in intake during P2 was then calculated as the percentage of ADFI reduction relative to P1 for each d of HS exposure. The percentage of ADFI reduction was averaged for all HSAL pigs per d of heat exposure and applied individually to the baseline of each pig in the TNPF treatment as we have previously described (Sanz Fernandez et al., 2015a, 2015b). The calculated amount of feed was evenly distributed and offered to the TNPF pigs three times daily (~0600, 1200, and 1800 h) in an attempt to minimize gorging induced postprandial shifts in metabolism. Ambient temperature was controlled but humidity was not governed and both parameters were recorded every 30 min by four data loggers (Lascar EL-USB-2-LCD, Erie, PA) distributed evenly in each room.

Production and Thermoregulation Measurements

Daily feed intake was measured during P1 and P2 as feed disappearance. Body weights were obtained at the beginning and the end of P1 and on d 7, 14, and 21 of P2. Rectal temperature (T_R) was measured with a calibrated digital thermometer (ReliOn, Waukegan, IL), skin temperature (T_S) was measured using a calibrated infrared thermometer (IRT207: The Heat Seeker 8:1 Mid-Range Infrared Thermometer, General Tools, New York, NY), and respiration rate (RR) was determined by counting flank movements during a 15 s interval and multiplied by 4 to obtain breaths/min. All thermal indices were recorded twice daily (0600 and 1800 h) and condensed into weekly AM and PM averages.

Blood Sampling and Analysis

Blood was obtained via jugular venipuncture (10 mL; BD vacutainers; Franklin Lakes, NJ; K3EDTA; EDTA) at 0600 h (following

thermoregulation measurements and prior to feeding) on d 1 of P1 (before dietary treatment initiation) and d 7 of P1, and at 0600 h on d 8, 15, and 21 of P2. Plasma samples were harvested by centrifugation at 4 °C and $2,500 \times g$, aliquoted and stored at -80 °C until further analysis. Plasma glucose was measured enzymatically using a commercially available kit (Wako Chemicals USA, Richmond, VA); the intra- and interassay coefficients of variation were 13.7 and 10.0%, respectively. An ELISA kit was used to determine plasma insulin (Mercodia Porcine Insulin ELISA; Mercodia AB; Uppsala, Sweden); the intra- and interassay CVs were 5.8 and 5.5%. Both assays were conducted following the manufacturer's instructions and were read using a microplate photometer (Hycult Biotech, Uden, Netherlands).

Tissue Collection and Fatty Acid Composition Analysis

At the conclusion of the experiment, pigs were euthanized via captive bolt followed by exsanguination. Abdominal visceral fat as well as inner and outer s.c. adipose from the nape of the neck were immediately collected, snap frozen in liquid nitrogen, and stored at -80 °C until analysis. Back fat thickness at the nape of the neck (above cervical vertebrae) was measured to the nearest 0.1 cm using a ruler.

Lipids from abdominal, inner s.c., and outer s.c. adipose depots (nape of the neck) were extracted and FA methyl esters were prepared and quantified by gas chromatography. Wet tissue lipid extraction was performed as previously described (Madron et al., 2002) and FA methyl esters were prepared by transmethylation (Christie, 1982) with modifications (Chouinard et al., 1999). Fatty acid methyl esters were quantified by a gas chromatograph (Varian GC system 3900, Agilent Technologies, Santa Clara, CA) equipped with a flame-ionization detector and an Agilent DB-23 cyanopropyl capillary column (60 m $\times$ 0.25 mm i.d. with 0.15- μ m thickness, Agilent Technologies, Santa Clara, CA). Initial oven temperature (50 °C) was held for 1 min then ramped at 25 °C/min to 175 °C and thereafter ramped at 4 °C/min to 230 °C, where it was held for 8 min. Injector and detector temperatures were maintained at 240 °C, and the split ratio was 100:1. Helium carrier gas flow rate through the column was 2 mL/min. Peaks in the chromatogram were identified and quantified using pure methyl ester standards gas liquid chromatography (GLC) 68D and GLC461. Chromatogram analysis was carried out

using Varian Star Chromatography Workstation Version 5.52. Iodine value was calculated using the following equation as previously described (Kellner et al., 2016):

$$\begin{aligned} \text{Iodine value} = & (\text{C16:1} \times 0.95) + (\text{C18:1} \times 0.86) \\ & + (\text{C18:2} \times 1.732) + (\text{C18:3} \times 2.616) \\ & + (\text{C20:1} \times 0.785) + (\text{C22:1} \times 0.723) \end{aligned}$$

Stearoyl-CoA desaturase indices for palmitic acid (C16:0), stearic acid (C18:0), and total were calculated using the following equations:

$$\begin{aligned} \text{SCD index} = & \frac{(\text{C14:1} + \text{C16:1} + \text{C18:1})}{(\text{C14:1} + \text{C16:1} + \text{C18:1} + \text{C14:0} \\ & + \text{C16:0} + \text{C18:0})} \end{aligned}$$

Adipose Tissue Moisture Content and Morphology Analysis

Adipose samples were weighed and then dried at 37.7 °C (Precision: Division of Jouan Inc., Winchester, VA) to a constant weight (96 h) to determine moisture content. To determine adipocyte cell size, frozen adipose tissue samples were sent to the University of Iowa Histology Research Laboratory for sectioning and hematoxylin and eosin staining. Microscopy was carried out using a microscope (DMI3000 B Inverted Microscope; Leica, Bannockburn, IL) with an attached 12-bit QICAM Fast 1394 camera (QImaging, Surrey, BC) to obtain four images per section using Q Capture Pro software (Surrey, BC, Canada). Raw images were converted to solid contrasting colors using Open Lab software (Perkin Elmer, Waltham, MA) and area was calculated using Image Pro Plus software (MediaCybernetics, Rockville, MD). All area measurements were condensed into single averages for each adipose depot per experimental unit.

Statistical Analysis

All data were analyzed using PROC MIXED (version 9.3, SAS Institute Inc., Cary, NC). Period 2 thermoregulatory and production data were analyzed with an autoregressive covariance structure with wk of experiment as the repeated effect. The model included treatment, wk, and their interaction as fixed effects; BW recorded at the beginning of P1 (prior to dietary treatment initiation) was used as a covariate. Plasma insulin was analyzed with a spatial power law covariance structure with d of the

experiment as the repeated effect and treatment, d, and their interaction as fixed effects; insulin levels from the plasma sample obtained at 0600 h of P1D1 (prior to dietary treatment initiation) were used as a covariate. Adipose tissue moisture content, back fat thickness, adipocyte area, and adipose FA content were analyzed using BW recorded at the beginning of P1 as a covariate. Preplanned orthogonal contrasts were conducted to evaluate differences among environmental treatments (i.e., TNAL vs. HS [HSAL, HSSO, and HSCr], TNPF vs. HS, and TNAL vs. TNPF pigs) and dietary supplements (i.e., HSAL vs. HSSO, and HSAL vs. HSCr pigs). Data are reported as LSmeans and statistical significance ($P \leq 0.05$) and tendency thresholds ($0.05 < P \leq 0.10$) were utilized for interpretation.

RESULTS

Thermoregulatory, Production, and Blood Indices

During P1, no treatment differences were detected for any thermoregulatory measurements. Although AM T_R was not different (Fig. 1A) during P2, HS increased AM RR and T_S (20 bpm and 1.91 °C, respectively; $P < 0.01$; Table 2) and PM T_R (Fig. 1B), RR, and T_S (1.13 °C, 48 bpm, and 3.51 °C, respectively; $P < 0.01$; Table 2) relative to TNAL controls. While AM and PM RR and T_R were not different, AM and PM T_S (1.49 °C and 1.18 °C, respectively; $P < 0.01$) were decreased in TNPF compared to TNAL controls during P2. Heat-stressed pigs fed SO had increased PM T_R relative to HSAL during wk 2 and 3 of P2 (0.36 °C and 0.44 °C, respectively; $P \leq 0.05$; Fig. 1B), but Cr did not affect thermal indices.

No dietary treatment differences were detected for ADFI, BW, or ADG during P1. During P2, HS decreased ADFI compared to TNAL pigs (32%; $P < 0.01$; Table 3). Similarly, ADG and final BW were decreased in HS treatments compared to TNAL pigs (42% and 7%, respectively; $P < 0.01$; Table 3); neither ADFI nor ADG were influenced by SO or Cr supplementation (Table 3). By experimental design, TNPF pigs had a similar magnitude and pattern of reduced ADFI and their ADG, BW and G:F variables did not differ from their HS counterparts. There was also no overall treatment effect on G:F ($P = 0.20$; Table 3).

During P1, no dietary treatment differences in circulating insulin or glucose were detected. Relative to TNAL pigs during P2, HS decreased circulating insulin (25%; $P < 0.01$); however, circulating insulin increased in HS pigs relative to TNPF controls

Figure 1. Effect of thermoneutral (TN) ad libitum (TNAL), TN pair-fed (TNPF), heat stress (HS) ad libitum (HSAL), HS sterculic oil (HSSO), and HS chromium (HSCr) on AM rectal temperature (T_R ; A) and PM T_R (B). Error bars represent SE for each wk during the study. The dashed line separates period (P) 1 from P2.

Table 2. Effects of period 2 treatment on body temperature indices. ^{a-d}Values with differing superscripts denote differences ($P \leq 0.05$) between treatments

Parameter	Treatments ¹					P								
	TNAL	TNPF	HSAL	HSSO	HSCr	SEM	Trt ²	Wk	Trt*Wk ³	TNAL vs. HS ⁴	TNPF vs. HS	TNAL vs. TNPF	HSAL vs. HSSO	HSAL vs. HSCr
T_R AM ⁵ , °C	39.69	39.62	39.73	39.74	39.69 ^c	0.07	0.82	<0.01	0.15	0.73	0.27	0.53	0.89	0.72
T_S AM ⁶ , °C	30.95 ^a	29.46 ^b	32.91 ^c	32.96 ^c	32.70 ^c	0.38	<0.01	0.88	<0.01	<0.01	<0.01	<0.01	0.92	0.69
RR AM ⁷ , bpm	57.54 ^a	50.93 ^a	76.26 ^b	77.13 ^b	77.87 ^b	2.41	<0.01	0.01	0.18	<0.01	<0.01	0.18	0.86	0.74
T_R PM ⁸ , °C	39.91 ^a	39.75 ^a	40.85 ^b	41.18 ^c	40.84 ^b	0.12	<0.01	<0.01	0.05	<0.01	<0.01	0.37	0.07	0.94
T_S PM ⁹ , °C	33.13 ^a	31.95 ^b	36.13 ^c	36.13 ^c	35.90 ^c	0.22	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.99	0.47
RR PM ¹⁰ , bpm	59.28 ^a	53.34 ^a	102.74 ^b	107.46 ^b	103.36 ^b	3.60	<0.01	<0.01	<0.01	<0.01	<0.01	0.25	0.36	0.90

¹Treatments: TNAL = thermoneutral (TN) *ad libitum*; TNPF = TN pair-fed; HSAL = heat stress (HS) *ad libitum*; HSSO = HS sterculic oil; HSCr = HS chromium

²Treatment

³Treatment by wk interaction

⁴All HS treatments

⁵Rectal temperature (T_R) at 0600

⁶Skin temperature (T_S) at 0600

⁷Respiration rate (RR) in breaths per minute (bpm) at 0600

⁸ T_R at 1800

⁹ T_S at 1800

¹⁰RR in bpm at 1800

Table 3. Effects of period 2 treatment on production parameters. ^{a-d}Values with differing superscripts denote differences ($P \leq 0.05$) between treatments

Parameter	Treatments ¹						<i>P</i>							
	TNAL	TNPF	HSAL	HSSO	HSCr	SEM	Trt ²	Wk	Trt*Wk ³	TNAL vs. HS ⁴	TNPF vs. HS	TNAL vs. TNPF	HSAL vs. HSSO	HSAL vs. HSCr
ADFI, kg/d	3.28 ^a	002.46 ^b	2.29 ^c	2.31 ^c	2.11 ^c	0.14	<0.01	<0.01	0.53	<0.01	0.20	<0.01	0.90	0.39
FBW ⁵ , kg	143.5 ^a	134.9 ^b	134.2 ^b	133.9 ^b	133.3 ^b	1.8	<0.01	-	-	<0.01	0.60	<0.01	0.91	0.75
ADG, kg/d	1.09 ^a	0.62 ^b	0.62 ^b	0.65 ^b	0.62 ^b	0.05	<0.01	<0.01	0.12	<0.01	0.85	<0.01	0.78	0.99
G:F	0.33	0.25	0.26	0.27	0.30	0.03	0.20	<0.01	0.08	0.07	0.39	0.03	0.94	0.39

¹Treatments: TNAL = thermoneutral (TN) ad libitum; TNPF = TN pair-fed; HSAL = heat stress (HS) ad libitum; HSSO = HS sterculic oil; HSCr = HS chromium

²Treatment

³Treatment by wk interaction

⁴All HS treatments

⁵Final BW

Figure 2. Effect of thermoneutral (TN) ad libitum (TNAL), TN pair-fed (TNPF), heat stress (HS) ad libitum (HSAL), HS sterculic oil (HSSO), and HS chromium (HSCr) on circulating insulin (A) and glucose (B) levels during period 2. Error bars represent SE for each treatment during the study. ^{a-d}Values with differing superscripts denote differences ($P \leq 0.05$) between treatments.

(33%; $P = 0.02$; Fig. 2A). Circulating insulin was not influenced by SO ($P = 0.12$), but increased with Cr supplementation relative to HSAL controls (0.13 vs. 0.10 µg/L; $P = 0.05$; Fig. 2A). During P2,

circulating glucose increased with HS compared to TNAL pigs (13%; $P < 0.01$), but it did not differ from TNPF controls ($P = 0.24$; Fig. 2B). Sterculic oil supplementation did not alter circulating glucose

relative to HSAL pigs ($P = 0.33$), but plasma glucose decreased with dietary Cr (8%; $P = 0.01$; Fig. 2B).

Fatty Acid Composition

There were marginal environmental effects on fatty acid composition in the three adipose depots evaluated (Supplementary Tables S1–S3). The primary dietary affect on fatty acid composition was the decrease in SCD products (C14:1, C16:1 and C18:1) in all three depots from the HSSO fed pigs. Consequently, the total saturated fatty acid and monounsaturated fatty acid content increased and decreased, respectively, in each of the adipose depots from the SO fed pigs (Table 4).

Back Fat Thickness, Adipocyte Area, and Adipose Moisture Content

Back fat thickness was not influenced by diet or environment ($P = 0.87$; Table 4). However, abdominal adipocyte size was decreased in TNPF pigs relative to TNAL controls (26%; $P = 0.02$; Table 4). In HSAL pigs, abdominal adipocyte size was increased compared to TNPF controls (37%; $P = 0.01$), but it did not differ compared to TNAL-fed pigs ($P = 0.74$; Table 4). Sterculic oil and Cr supplementation decreased abdominal adipocyte size relative to HSAL (22% and 24%, respectively; $P \leq 0.04$; Table 4). No differences in adipocyte size were detected at inner and outer s.c. adipose depots (Table 4).

Regardless of SO and Cr supplementation, HS increased moisture content in the abdominal (30%; $P = 0.07$) and inner s.c. (16%; $P = 0.05$; Table 4 and Fig. 3) adipose depots relative to TNAL controls. Similarly, TNPF pigs had increased moisture content in the outer s.c. (23%; $P = 0.01$; Table 4 and Fig. 3) adipose depot relative to TNAL controls; however, moisture content in outer s.c. adipose tissue was decreased in HS treatments compared to TNPF (11%; $P = 0.04$; Table 4 and Fig. 3).

DISCUSSION

Despite aggressive heat abatement strategies, HS remains a major economic burden to the U.S. swine industry with an estimated \$900 million in annual losses during the warm summer months (Pollmann, 2010). Sources of reduced revenue include slower growth rates, inefficient facility utilization, increased health care costs, inconsistent market weights, mortality, and altered carcass

composition (Baumgard and Rhoads, 2013; Ross et al., 2017). In addition, post-harvest adipose tissue is softer (also referred to as “flimsy fat”) from pigs marketed during the summer, and this creates processing and handling complications (Dr. R. Johnson, Smithfield Farmland, I. A. Denison, personal communication).

In this experiment, pigs allocated to the three HS treatments experienced a significant heat load, which was reflected by marked thermoregulatory responses (Table 2). Interestingly, supplementing SO increased T_r relative to the other two HS treatments during the last two weeks of P2. The reduced desaturase index at all adipose depots in HSSO pigs suggested SO supplementation attenuated SCD activity, which is responsible for inserting a double bond at the 9th position of myristic, palmitic, and stearic acids. Importantly, this enzyme has also been implicated in thermoregulation as SCD knockout mice have impaired thermoregulatory capacity and become critically hypothermic when housed in cold environments (Lee et al., 2004). Disrupting SCD with dietary SO also impaired thermoregulation in this experiment and identifying whether or not SCD could be manipulated to help pigs maintain a healthy body temperature during HS is of interest.

Heat stress markedly decreased ADFI, ADG, and final BW. Interestingly, while ADG and G:F from wk 2 to 3 (data not shown) plateaued in pigs from the HSAL and HSSO treatments, both parameters continued to increase (30% from wk 2 to 3) in HSCr pigs. Improvements in ADG and G:F have been observed in pigs fed Cr (Lindemann et al., 1995; Hung et al., 2010; Sales and Jancik, 2011; Mayorga et al., 2016). While evaluating the effect of dietary supplements on traditional production parameters was not our primary objective, it would be interesting to investigate ADG and G:F over a longer duration to determine if HSCr pigs maintained their improvements in these economically important phenotypes.

The primary objectives of this experiment were to investigate the effects of HS and insulin sensitization on carcass fat characteristics. Developmentally, the inner portion of s.c. adipose is the most recently synthesized (Fortin, 1986), so we separated this particular depot into outer (older) and inner (newest) sections because we hypothesized that the inner would be most responsive to a 21-d environmental and dietary intervention. Despite inconsistencies with respect to how HS influences adipose FA composition (Kloareg et al., 2005; White et al., 2008; Kellner et al., 2016), the potential for heat-induced FA unsaturation via increased SCD activity

Table 4. Effects of period 2 treatment on backfat thickness and fatty acid (FA) profile, adipocyte area, and moisture content of abdominal, inner, and outer s.c. adipose tissue depots during the study. ^{a-d}Values with differing superscripts denote differences ($P \leq 0.05$) between treatments

	Treatments ¹						<i>P</i>					
Parameter	TNAL	TNPF	HSAL	HSSO	HSCr	SEM	Trt ²	TNAL vs. HS ³	TNPF vs. HS	TNAL vs. TNPF	HSAL vs. HSSO	HSAL vs. HSCr
Back fat thickness (cm)	0003.61	3.73	3.97	3.72	3.63	0.26	0.88	0.60	0.87	0.76	0.50	0.39
Abdominal												
Adipocyte area (µm ²)	3299 ^{bc}	2431 ^a	3796 ^c	2970 ^{ab}	2888 ^{ab}	266	0.01	0.74	0.01	0.02	0.04	0.02
Moisture content (%)	5.90	7.33	7.67	8.76	6.57	0.80	0.15	0.07	0.72	0.23	0.34	0.34
IV ⁴	54.80	55.52	56.46	52.44	56.36	1.57	0.40	0.88	0.80	0.74	0.09	0.97
SCDI (14,16,18) ⁵	0.45 ^b	0.44 ^b	0.41 ^b	0.36 ^a	0.43 ^b	0.01	<0.01	0.01	0.03	0.69	0.02	0.49
.....g/100 g fatty acids												
Chain length <16	1.35	1.34	1.50	1.49	1.49	0.08	0.38	0.12	0.10	0.95	0.96	0.92
C16:0 and C16:1	27.37	26.99	27.21	26.78	27.83	0.64	0.82	0.90	0.70	0.67	0.64	0.49
Chain length >16	69.11	69.06	69.27	69.74	69.02	0.65	0.94	0.76	0.71	0.96	0.62	0.79
SFA ⁶	46.65 ^a	46.53 ^a	47.79 ^a	52.26 ^b	47.38 ^a	1.40	0.05	0.14	0.12	0.95	0.04	0.84
MUFA ⁷	38.15 ^c	36.80 ^{bc}	34.24 ^b	29.97 ^a	35.91 ^{bc}	1.14	<0.01	<0.01	0.02	0.41	0.02	0.31
PUFA ⁸	13.03	14.07	15.96	15.78	15.05	0.80	0.07	<0.01	0.11	0.36	0.88	0.43
Inner s.c.												
Adipocyte area (µm ²)	2328	2046	2255	2019	2235	183	0.71	0.46	0.55	0.27	0.39	0.94
Moisture content (%)	9.8	11.03	10.81	11.90	11.39	0.67	0.27	0.05	0.66	0.20	0.26	0.54
IV	68.65	68.00	68.05	64.32	69.30	1.40	0.13	0.38	0.63	0.74	0.07	0.53
SCDI (14,16,18)	0.54 ^c	0.53 ^{bc}	0.51 ^b	0.46 ^a	0.52 ^{bc}	0.01	<0.01	<0.01	0.02	0.40	<0.01	0.62
.....g/100 g fatty acids												
Chain length <16	1.09	1.19	1.19	1.26	1.25	0.06	0.29	0.04	0.54	0.24	0.40	0.53
C16:0 & C16:1	22.86	22.98	22.68	23.61	23.41	0.61	0.80	0.60	0.72	0.89	0.29	0.40
Chain length >16	73.09	72.19	73.30	72.38	73.21	0.64	0.64	0.86	0.31	0.33	0.32	0.92
SFA	35.34 ^a	35.90 ^a	37.41 ^a	41.69 ^b	36.98 ^a	1.22	0.01	0.02	0.06	0.75	0.02	0.81
MUFA	42.48 ^c	40.86 ^{bc}	39.26 ^b	35.41 ^a	40.09 ^b	0.82	<0.01	<0.01	0.01	0.17	<0.01	0.48
PUFA	19.21	19.60	20.51	20.16	20.79	0.75	0.58	0.16	0.32	0.72	0.74	0.80
Outer s.c.												
Adipocyte area (µm ²)	2289	2143	2049	2136	1978	091	0.18	0.03	0.46	0.29	0.47	0.63
Moisture content (%)	12.15	14.98	13.05	13.09	13.85	0.68	0.07	0.14	0.04	0.01	0.96	0.41
IV	71.12	70.56	71.52	69.99	71.53	1.05	0.81	0.93	0.71	0.71	0.31	0.99
SCDI (14,16,18)	0.57 ^c	0.55 ^{bc}	0.54 ^b	0.51 ^a	0.56 ^{bc}	0.01	<0.01	<0.01	0.15	0.12	0.01	0.17
.....g/100 g fatty acids												
Chain length <16	1.10	1.24	1.17	1.22	1.09	0.06	0.36	0.41	0.27	0.12	0.60	0.40
C16:0 and C16:1	22.82	22.72	22.05	22.42	21.53	0.49	0.35	0.16	0.21	0.88	0.59	0.46
Chain length >16	72.95	72.14	73.76	73.30	74.16	0.59	0.16	0.25	0.02	0.33	0.59	0.63
SFA	33.04 ^a	33.69 ^a	34.32 ^a	36.48 ^b	33.35 ^a	0.78	0.03	0.08	0.27	0.56	0.06	0.39
MUFA	43.92 ^d	41.69 ^{bc}	40.95 ^b	38.55 ^a	42.46 ^c	0.54	<0.01	<0.01	0.11	0.01	<0.01	0.06
PUFA	19.91	20.71	21.71	21.90	20.98	0.64	0.20	0.04	0.28	0.38	0.83	0.42

¹Treatments: TNAL = thermoneutral (TN) ad libitum; TNPF = TN pair-fed; HSAL = heat stress (HS) ad libitum; HSSO = HS sterculic oil; HSCr = HS chromium

²Treatment

³All HS treatments

⁴Iodine value

⁵Stearoyl-CoA desaturase index

⁶Total saturated FA

⁷Total monounsaturated FA

⁸Total polyunsaturated FA

Figure 3. Effect of thermoneutral (TN) ad libitum (TNAL), TN pair-fed (TNPF), and all heat stress (HS) treatments combined on adipose tissue moisture content during period 2. Error bars represent SE for each treatment.

represents a possible underlying cause for the aforementioned “flimsy fat” phenotype. The very name suggests there would be an overall increase in FA unsaturation, which would reduce firmness due to reduced melting points of MUFA and PUFA. Furthermore, we hypothesized that the heat-induced increased circulating insulin would upregulate SCD abundance and activity, a scenario which should promote FA desaturation. However, cellular membranes generally become more fluid-like at higher ambient temperatures (Hazel, 1995) and therefore it would be biologically advantageous to employ mechanisms to increase FA saturation in order to maintain membrane integrity and plasticity. In agreement with this homeoviscous adaptation concept, adipose from HSAL had decreased MUFAs, but numerically increased SFA in all adipose depots compared to TNAL. Heat-induced increases in saturated FA content in pigs has also been observed elsewhere (Lefaucheur et al., 1991; Katsumata et al., 1995; Kouba et al., 1999; Kloareg et al., 2005). Furthermore, pigs reared in colder conditions exhibit a higher degree of FA unsaturation (MacGrath et al., 1968; Fuller et al., 1974; Le Dividich et al., 1987; Lefaucheur et al., 1991). Therefore, the summer affiliated “flimsy fat” phenotype occurring as a result of increased desaturation appears unlikely as the data reported herein conforms with the homeoviscous adaptation concept.

We also evaluated adipocyte size because it was thought to be associated with adipose tissue firmness (Mendizabal et al., 2004). Due to insulin’s ability to stimulate adipocyte proliferation (Geloan et al., 1989), we hypothesized heat-induced insulin response would result in more but smaller adipocytes. Although HS increased circulating insulin, it

had little or no effect on adipocyte size, but feed restriction and SO and Cr supplementation decreased adipocyte size in the abdominal adipose depot, while no differences were detected at inner or outer s.c. adipose tissue locations. Previous studies have reported HS-induced increases in back fat adipocyte diameter in pigs (Rinaldo and Le Dividich, 1991) and cold stress-induced decreases in epididymal adipocyte diameter in rats (Cherqui et al., 1979). Differences in experimental design (e.g., pattern, extent, adipose depot type, and magnitude of HS) may contribute to our lack of observed changes.

Interestingly, we observed HS-induced increased moisture content at all adipose depots relative to TNAL controls. Although the exact mechanisms for this are not clear, water content has been shown to be inversely related to s.c. adipose tissue firmness in boars and barrows (Wood et al., 1985), thus increased moisture content may at least partly contribute to soft carcass fat phenotypes observed during the summer months. This may be partially due to decreased feed intake, as pair-fed animals also had increased moisture content in outer s.c. adipose tissue relative to TNAL controls. Furthermore, obese humans administered a low calorie diet have increased water content of abdominal adipose tissue (Laaksonen et al., 2003). However, abdominal and s.c. adipose tissue water content was not affected by nutrient restriction in the current study, indicating a potential interaction of feed intake, environment, and adipose depot location. Further investigation into mechanisms and biological reasons for increased adipose water content during HS and nutrient restriction warrant additional research. Increased adipose tissue

blood flow could also represent a likely explanation for increased adipocyte moisture content as obese humans have lower adipose tissue perfusion compared to lean individuals (Blaak et al., 1995) and this is improved with low calorie diet administration and weight loss (Blaak et al., 1995; Barbe et al., 1997). Regardless of the potential mechanism, increased carcass adipose moisture content is not trivial as it directly affects adipose firmness and, ultimately, carcass processing. Whether or not increased adipose moisture content as a result of HS can be prevented will require a more comprehensive investigation.

CONCLUSION

In summary, despite the occurrence of the “flimsy fat” phenotype in response to HS, the overall degree of FA unsaturation was not increased in HS pigs at abdominal, inner, and outer s.c. adipose depots. Adipocyte size was not affected by HS, but was impacted by nutrient restriction as well as both Cr and SO. Interestingly, all HS treatments exhibited increased adipose moisture content at all three adipose depots, but this may be partly attributed to reduced ADFI. Whether or not increased moisture content is linked to the altered carcass fat quality observed during the summer months is of scientific and practical interest, but will require more detailed investigation.

SUPPLEMENTARY DATA

Supplementary data are available at *Animal Frontiers* online.

Conflict of interest statement. None declared.

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