RESEARCH REPORT
PORK QUALITY

Title: Impact of Seasonal Heat Stress on Fatty Acid Composition and Pork Fat Quality – **NPB #13-080**

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Industry Summary:

Heat stress (HS) remains one of the most costly factors in the swine industry and is responsible for increased mortality, altered carcass compositions, reduced reproductive ability, and slower growth rates in pork production during the warm summer months. This project was conducted to elucidate the causes of one particular altered carcass trait in response to HS, "flimsy fat", which negatively affects the processing and final product value. Project objectives were to determine effects of environmental HS and dietary fat sources on adipose characteristics as well as elucidating the biological mechanisms by which HS negatively affects carcass fat quality. The data in this project demonstrate that HS does not significantly influence fatty acid composition of adipose tissue or adipocyte cell size. However, HS does increase adipose tissue moisture content (31, 16, and 10% in the abdominal, inner, and outer subcutaneous adipose depots, respectively), but this appears to be partially due HS-induced reduced feed intake. Collectively, the "soft" fat that occurs during HS is not due to fatty acid composition changes or adipocyte cell size but the increased adipose moisture content (discovered in these experiments) is likely linked to the "flimsy fat" phenotype. Future investigations will be required to firmly establish how HS alters adipose tissue moisture content and to determine of a mitigation factor can be developed to ameliorate it.

Key Findings

- HS did not alter fatty acid composition in any adipose tissue depot evaluated.
- All heat-stressed pigs and pair-fed controls exhibited increased adipose tissue moisture content.
- Inhibiting fatty acid desaturation impairs an animal's thermoregulatory system.
- There were no interactions between dietary fat source and production parameters during HS.
- HS decreased apparent total track digestibility of dietary lipids, but had no impact on true total track digestibility of dietary lipids.
- Modifying insulin sensitivity using chromium or sterculic oil did not improve production parameters during heat stress.

Keywords: Adipose tissue, Heat stress, Swine

Scientific Abstracts:

Experiment 1

Heat stress results in major losses to the pork industry through reduced growth performance and possibly carcass fat quality. The objective was to investigate the effect of heat stress on the pig's response to dietary fat in terms of growth performance, dietary lipid digestion, and pork fat quality over a 35 d finishing period. A total of 96 barrows (PIC 337 × C22/29) with an initial BW of 100.4 ± 1.2 kg were randomly allotted to 1 of 9 treatments arranged as a 3 × 3 factorial: [TN (thermonetural: constant 24°C; ad libitum access to feed), PFTN (pair-fed thermoneutral: constant 24°C; limit-fed based on previous HS daily feed intake), or HS (heat stress: cyclical 28°C nighttime, 33°C-35°C daytime; ab libitum access to feed)] and diet [a corn-soybean meal based diet with 0% added fat (CNTR), 3% added tallow (3%TAL; iodine value = 41.8), or 3% added corn oil (3%CO; iodine value = 123.0)]. Pigs were individually housed to track intake and to allow for jowl fat biopsies. Titanium dioxide was included as an indigestible marker at 0.4%. Fecal samples were collected on d 17 (~ 114 kg). True total track digestibility (TTTD) (%) of acid hydrolyzed ether extract (AEE) was calculated by correcting apparent total track digestibility (ATTD) of AEE for endogenous fat losses at 20 g of AEE/kg of dry matter intake. Data were analyzed using PROC MIXED with environment and dietary treatment as fixed effects, and replicate (2 replicates of 48 barrows) as a random effect. Rectal temperature (HS = 39.0, TN = 38.1, PFTN = 38.2°C) and respiration rates (HS = 78, TN = 36, PFTN = 34 bpm) increased due to HS ($P < 0.001$). HS decreased ADFI (27.8%; $P < 0.001$). ADG (HS = 0.72, TN = 1.03, PFTN = 0.78 kg/d; $P < 0.001$), and G:F (HS = 0.290, TN = 0.301, PFTN = 0.319; $P < 0.01$) also reduced by HS. G:F but not ADG or ADFI tended to be influenced by dietary treatment (CNTR =

0.292, 3%TAL = 0.303, 3%CO = 0.314 g/100 g; $P \leq 0.07$). Exposure to HS did not impact IV at market (HS = 69.2, TN = 69.3, PFTN = 69.8 g/100 g; $P = 0.62$). Carcass IV increased with increasing degree of unsaturation of the dietary fat (CNTR = 68.5, 3%TAL = 68.2, 3%CO = 71.5 g/100 g; $P < 0.001$). HS tended to have the lowest ATTD of AEE (HS = 59.0, TN = 60.2, PFTN = 61.4%, $P = 0.055$). Inclusion of dietary fat, and a source that was unsaturated increased ATTD of AEE (CNTR = 41.6, 3%TAL = 67.9, 3%CO = 71.2%, $P < 0.001$). TTTD of AEE of 3%CO-based diets was higher (99.3%) than that of CNTR (97.3%) and 3%TAL-based diets (96.3%; $P = 0.01$). Environment had no impact of TTTD of AEE ($P = 0.12$). There was no interaction between HS and diet ($P \geq 0.19$) on carcass IV. In conclusion, HS impaired growth performance but not carcass IV or the response to dietary fat. IV was affected by dietary fat source.

Experiment 2

Heat stress (HS) negatively impacts several production variables in swine, including carcass fat quality. Study objectives were to evaluate insulin's role (via feeding insulin sensitizing compounds) in decreasing fat quality during HS. Forty crossbred barrows (113±9 kg BW) were randomly assigned to 1 of 5 treatments during 2 experimental periods: 1) thermoneutral (TN) *ad libitum* feed intake control (TNAL), 2) TN pair-fed control (TNPF), 3) HS *ad libitum* (HSAL), 4) HS *ad libitum* with dietary sterculic oil (HSSO; 13 g/d), and 5) HS *ad libitum* with dietary chromium propionate (HSCr; 0.5 mg/d; Kemin Industries, Des Moines, IA). During period 1 (7 d), all pigs were exposed to TN conditions (23±3°C, 68±10% RH) and fed *ad libitum*. During period 2 (21 d), HSAL, HSSO, and HSCr pigs were fed *ad libitum* and exposed to cyclical (HS) conditions (28 to 33°C, 58±10% RH). The TNAL and TNPF pigs remained in TN conditions and were fed *ad libitum* or pair-fed to their HSAL counterparts (to eliminate the confounding effect of dissimilar nutrient intake), respectively. Rectal temperature (T_r), respiration rate, and skin temperature were increased (0.9°C, 37 bpm, and 2.5°C, respectively) in HS pigs. Interestingly, HSSO increased T_r relative to HSAL and HSCr (0.40 and 0.42°C, respectively) during the last week of period 2 ($P < 0.05$). HS decreased feed intake and ADG compared to TNAL (2.43 vs. 3.26 kg/d and 0.74 vs. 1.09 kg/d, respectively; $P < 0.01$) and neither variable was affected by SO or Cr supplementation. Moisture content tended to be increased in pigs from all HS treatments compared to TNAL controls in abdominal (7.7 vs. 5.9%; $P < 0.09$) and inner subcutaneous adipose tissue (11.4 vs. 9.8%; $P < 0.06$) depots. Interestingly, TNPF pigs had or tended to have increased adipose tissue moisture content in abdominal (7.3 vs. 5.9%; $P < 0.01$), inner subcutaneous (11.0 vs. 9.8%; $P < 0.07$), and outer subcutaneous (14.9 vs. 12.1%; $P < 0.01$) depots compared to TNAL pigs. HS had little or no effect on fatty acid (FA) composition of abdominal, inner, and outer subcutaneous adipose tissue depots. Feeding SO decreased the desaturase index in the abdominal (0.36 vs. 0.43; $P < 0.01$), inner subcutaneous (0.46 vs. 0.52; $P < 0.01$), and outer subcutaneous (0.51 vs. 0.55; $P < 0.01$) adipose tissue. In

summary, HS did not alter FA composition in any adipose depot, but both TNPF and HS markedly increased adipose tissue moisture content.

Introduction:

Heat stress markedly affects a plethora of production variables and is therefore one of the costliest issues for American pork producers. In addition, pigs reared and eventually harvested in the summer typically have decreased carcass fat quality, which causes a variety processing problems. Although this summer-induced carcass phenotype has been documented for years, the causes and mechanisms responsible for it are not well defined.

Carcass fat quality (i.e. "rigidity") is in part determined by cellular fatty acid composition. Cell membranes are made up of fatty acids and the extent of fatty acid saturation influences the membrane's fluidity characteristics. Differing fatty acids have widely different melting points, so, depending upon the temperature, the cell can strategically select or modify fatty acids to maintain proper membrane fluidity properties (Hazel, 1995). In environmental physiology this concept is referred to as homeoviscous adaptation and it suggests when animals are cold-stressed they would increase the cellular content of polyunsaturated fatty acids and when they are heat-stressed they would increase the content of saturated fatty acids. However, despite the enormous economic impact of heat stress on the swine industry, very little is known about how heat stress alters adipose fatty acid composition in the finishing pig. There are inconsistencies in the literature as some suggest heat stress increases fatty acid desaturation (White et al., 2008), while others do not (Kloareg et al., 2007). However, it is likely that these contradictions are related to a variety of trial design issues that primarily center on environmental conditions that did not mimic commercial heat load patterns (i.e. the maximum and minimum daily temperatures were too low). Regardless, input from pork processors indicates carcass fat consistently becomes "flimsy" during the warm summer months, and this suggests (because of fatty acid melting points) the membrane and cellular content of polyunsaturated fatty acids content actually *increases* instead of *decreases* during heat stress as homeoviscous adaptation predicts.

The primary process by which cells regulate fatty acid saturation properties is via the steroyl-CoA desaturase (SCD) system. This enzyme inserts a double bond at the 9^{th} position of myristic (C14:0), palmitic (C16:0), stearic (C18:0) and vaccenic (C18:1 t-11) acids and thus converts saturated fatty acids into mono- and polyunsaturated fatty acids. The activity and quantity of SCD is regulated by insulin and thus when insulin action is enhanced the SCD system is increased (Dobrzyn et al., 2010). Despite heat-stressed pigs having decreased nutrient intake and reduced growth, we have discovered that heat-stressed pigs have increased insulin parameters (Baumgard and Rhoads, 2012). This is energetically abnormal because insulin is a potent anabolic hormone and heat stress is a catabolic condition. Regardless, we hypothesize that heat-induced increased insulin stimulates

SCD, which increases membrane content polyunsaturated fatty acids and ultimately causes poor carcass fat quality from pigs finished during the warm summer months.

Adipocyte cell size also contributes to fat "rigidity" as small fat cells are more "pliable" while larger adipocytes are more rigid (Mendizabal et al., 2004). Effects of heat stress on adipocyte cell size in finishing pigs have not been extensively evaluated. However, insulin is a potent adipocyte proliferation signal (i.e. it causes cells to divide and increase in number) and we hypothesize that heat-induced insulin action increases adipocyte proliferation. This scenario would increase the number of fat cells while simultaneously decreasing the adipocyte size and we suspect this may partially explain the poor fat quality from heat-stressed pigs.

During heat stress, increasing the amount of saturated fatty acids in carcass lipid via changes in the diet could be a practical strategy to reduce the "flimsiness" and increase the quality of carcass fat. It has been understood for more than 75 years that the fat content of the pig's carcass is influenced by the nature of dietary fat (Ellis and Zeller, 1930; Banks and Hilditch, 1932). Further, in thermoneutral conditions the composition of dietary fat influences the fatty acid composition and quantity of carcass fat (Shorland and De La Mare, 1945; Brooks, 1971; Kellner and Patience, 2012). However, whether or not increasing the saturated fatty acid content of the diet can increase the saturated fatty acid content of carcass lipid during heat stress has not been extensively evaluated.

Fatty acid composition provides insight into the chemistry of a fat and is a predictor of its oxidative stability and firmness. Iodine value (IV) is related to the fatty acid profile and the higher the degree of unsaturation in the fat, the higher the IV will be (Kyriakidis and Katsiloulis, 2000). Iodine values can be determined directly by titration or estimated from the fatty acid profile (AOAC, 2006) (Ham et al., 1998). Iodine value (IV) is calculated by the following equation: (IV) = [C16:1] X 0.95 + [C18:1] X 0.86 + [C18:2] X 1.732 + [C18:3] X 2.616 + [C20:1] X 0.785 + [C22:1] X 0.723; brackets indicate concentrations (AOCS, 1998). The IV is a reflection of the amount of double bonds (unsaturation) within fatty acids and therefore is a predictor of carcass fat firmness (Benz et al., 2011). While specific fatty acids appear to be most closely related to meat quality issues, IV is used as one measure of the quality of carcass fat (Wood et al., 2003).

Objectives:

Our overall aim in this study was to determine the effects of heat stress during the finishing period on carcass fatty acid profile, IV, adipocyte cell size, and belly quality. In addition, we also planned to elucidate the biological mechanisms by which heat stress negatively affects carcass fat quality.

Materials & Methods:

Materials and methods for experiment 1

Barrows (n=96; two replicates of 48 each) were placed on 1 of 9 treatments arranged as a 3 × 3 factorial design for 35 days from 90 kg to market (125 kg). Factor 1 was the environmental conditions: 1) control – thermoneutral (TN) with *ad libitum* access to feed, 2) heat stressed (HS) - a diurnal heating pattern in an attempt to mimic commercial conditions with *ad libitum* access to feed, and 3) pair-fed thermoneutral (PFTN) – same as control but pigs received the same daily feed allowance as that consumed by the heat-stressed pigs from the previous day. The third environmental treatment allowed us to differentiate between the metabolic effects of heat stress versus the effect of the lower feed intake observed in heat-stressed pig. Factor 2 was the dietary treatments: 1) standard corn-soybean meal diet serving as the dietary control (CNTR), 2) control with 3% added tallow (3%TAL; IV ~40), and 3) control with 3% added corn oil (3%CO; IV ~120). The reason for the three diets was to show the positive or negative impact of additional dietary fat and the degree of fat unsaturation on the quality and composition of carcass fat under heat stress conditions. Adding corn oil directly rather than through DDGS provided a more controlled experimental circumstance. Pigs were housed in individual pens, so daily feed intake, and thus daily fatty acid intake, were recorded. This was a critical part of the experimental design; when pigs are housed in groups, feed intake is measured as an average of that group, with a wide range of feed intake among the pigs. In this experiment, the exact feed intake of individual pigs was known, so determining the relationship between dietary fat and carcass fat was more precise.

Two rooms at the ISU Swine Nutrition Farm were used for this experiment. One room was maintained within a thermoneutral temperature zone (24°C) by using air conditioning in the summer and heating in the winter. The heat stress room followed a diurnal pattern of heat stress that reflected the 24-hour pattern of barn temperatures observed in July and August in the Midwest (cyclical 28°C nighttime, 33°C d 0 to 7, 33.5°C d 7 to 14, 34°C d 14 to 21, 34.5°C d 21 to 28, 35°C d 28 to 35 daytime). Each room consisted of 24 individual pens; thus the thermoneutral room housed the barrows assigned to the control and the pair-fed thermoneutral (PFTN) treatments. Across two replicates, there were 24 control pigs and 24 PFTN pigs. This therefore provided 8 pigs per dietary treatment per environmental treatment. The heat stress room also contained 24 individual pens and provided 16 pigs across each of the dietary treatments. The diets (Table 1A) contained 0.40% titanium dioxide as a digestibility marker. Feces were collected on d 17 to determine apparent total track digestion (ATTD; %) of acid hydrolyzed ether extract (AEE). True total track digestibility (TTTD; %) of AEE was calculated by correcting ATTD of AEE for endogenous fat losses at 20 g of AEE/kg of dry matter intake. Subcutaneous fat samples were collected from the jowl at d 7 and d 21. These adipose samples were assayed for total fatty acid profile by gas chromatography. IV was determined from the fatty acid profile (AOCS, 1998). At 125 kg (d 35), the pigs were harvested at the JBS plant in Marshalltown, IA, where samples of jowl fat were collected. In

addition, bellies were collected to measure thickness, weight, temperature, color (Minolta), and firmness (durometer), and were also subjected to a subjective belly firmness test. Temperature had a very significant effect on belly firmness and was recorded. All of these samples were assayed for total fatty acid profile by gas chromatography, with IV calculated according to the equation developed by the AOAC as well as for IV by titration (AOAC, 1998).

Materials and methods for experiment 2

Animals and Experimental Design

Iowa State University Institutional Animal Care and Use Committee approved all procedures involving animals, which took place at the Iowa State University Swine Nutrition Farm research facility. Forty crossbred male pigs (113±20 kg body weight) were randomly assigned to one of five treatments during two experimental periods: 1) Thermoneutral *ad libitum* control (TNAL), 2) Thermoneutral pair-fed control TNPF, 3) Heat stress *ad libitum* (HSAL), 4) Heat stress *ad libitum* with sterculic oil supplementation (HSSO), 5) Heat stress *ad libitum* with chromium supplementation (HSCr). There were no differences in starting body weight among the five treatments after random assignment. During period 1 (7 d), all pigs were exposed to TN conditions (23±3°C, 68±10% RH) and fed *ad libitum*. During period 2 (21 d), HSAL, HSSO, and HSCr pigs were fed *ad libitum* and exposed to cyclical (HS) conditions (28 to 33°C, 58±10% RH). The TNAL and TNPF pigs remained in TN conditions and were fed *ad libitum* or pair-fed to their HSAL counterparts (to eliminate the confounding effect of dissimilar nutrient intake), respectively. The period 1 daily FI was averaged for each pig and used as a baseline. For each HSAL pig, the decrease in FI during period 2 was calculated as the percentage of FI reduction relative to period 1 for each day of HS exposure. This percentage of FI reduction was averaged for all the HSAL pigs per day of exposure and applied individually to the baseline of each TNPF pig. The calculated amount of feed was offered to the TNPF pigs three times daily (~0600, 1200, and 1800 h) in an attempt to minimize post-prandial shifts in metabolism. All pigs were fed a standard industry diet consisting mainly of corn and soybean meal formulated to meet or exceed nutrient requirements ((30)NRC, 2012). Pigs were individually housed in production-style crates in 1 of 2 environmental chambers/rooms with a 12h:12h light-dark cycle. 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) in each room and then condensed into averages for each time point. Rectal temperature (°C; T_R) was measured with a calibrated digital thermometer (ReliOn, Waukegan, IL, USA), skin temperature (°C; 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 in 15 seconds and multiplying that figure by four. All indices were recorded twice daily (0600 and

1800h) and condensed into daily AM and PM averages. Body weights (BW) were collected at the beginning and the end of period 1 and on days 7, 14, and 21 of period 2.

Tissue Collection

At the conclusion of the experiment, pigs were humanely euthanized by captive-bolt, desired tissues were collected (pancreas, liver, longissimus dorsi, abdominal fat, inner and outer subcutaneous adipose from the nape of the neck), snap frozen, and stored in -80°C until analysis.

Diet Administration

Diet treatments were administered to each pig daily during the entirety of the experiment, beginning on the first day of period 1. Each day, all TNAL, TNPF, and HSAL were fed 30 grams of cookie dough daily (Do-Biz Foods, LLC, Ames, IA). The HSSO pigs received a homogenized sample of seeds from *Sterculia foetida* tree and cookie dough (13 g of sterculic seeds and 30 g of cookie dough per day). The HSCr pigs received a homogenized sample of KemTRACE Chromium Propionate (Kemin Agri Foods North America Inc., Des Moines, IA) and cookie dough (0.5 mg chromium and 30 g cookie dough per day).

Blood Sampling and Blood Parameter Analysis

Blood (10 mL) was obtained on all pigs via jugular venipuncture (BD® vacutainers; Franklin Lakes, NJ; K3EDTA; EDTA) on d 1 (before diet treatment administration) and d 7 of period 1, d 1 of period 2 after the first day of HS, and before the heat load on days 2, 8, 15, and 21 of period 2. Plasma samples were harvested by centrifugation at 4°C and 2500 x *g*, aliquoted and stored at -80°C until further analysis. Plasma glucose and non-esterified fatty acids (NEFA) concentrations were measured enzymatically using commercially available kits (Wako Chemicals USA, Richmond, VA). The intra- and inter-assay coefficients of variation were 15.4 and 11.3%, and 14.3 and 14.5% for glucose and NEFA, respectively. An ELISA kit was used to determine plasma insulin (Mercodia Porcine Insulin ELISA; Mercodia AB; Uppsala, Sweden) and lipopolysaccharide binding protein (LBP) concentrations (Hycult Biotech, Uden, Netherlands), following the manufacturer's instructions. The intra- and inter-assay coefficients of variation were 5.8 and 5.5%, and 32.9 and 26.8% for insulin and LBP, respectively. These procedures were scaled down and conducted in 96 well microplates (Rainin Instrument LLC, Oakland, CA) and read using a microplate photometer (SpectraMax Plus, Molecular Devices, Silicon Valley, CA).

Fatty Acid Composition and Analysis

Lipids from abdominal, inner subcutaneous, and outer subcutaneous adipose depots were extracted and fatty acid methyl esters were prepared and quantified by gas chromatography. Wet tissue lipid extraction was

performed as previously described (Pogge et al., 2014) and fatty acid methyl esters were prepared by transmethylation (Christie, 1982) with modifications (Chouinard et al., 1999). Fatty acid methyl esters were quantified by using 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 × 0.25 mm i.d. with 0.15-µm 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 (Pogge *et al*. 2014). Chromatogram analysis was carried out using Varian Star Chromatography Workstation Version 5.52.

Iodine value was calculated: (C16:1 * 0.95) + (C18:1 * 0.86) + (C18:2 * 1.732) + (C18:3 * 2.616) + (C20:1 * 0.785) + (C22:1 * 0.723). Delta 9 (Δ^9) desaturase index for palmitic acid (C16:0) was calculated: (C16:1) / (C16:1 + C16:0). Δ^9 desaturase index for stearic acid (C18:0) was calculated: (C18:1) / (C18:1 + C18:0). Total Δ^9 desaturase index was calculated: (C14:1 + C16:1 + C18:1) / (C14:1 + C16:1 + C18:1 + C14:0 + C16:0 + C18:0). The index of atherogenicity was calculated: (C12:0 + (C14:0 * 4) + C16:0) / (Total MUFA + Total PUFA).

Adipose Tissue Moisture Content Analysis

An initial weight measurement was taken on each adipose tissue sample and then incubated at 37.7°C (Precision: Division of Jouan Inc., Winchester, VA) for 96 hours. After the incubation period, a final mass measurement was taken and was subtracted from the initial mass measurement to calculate moisture content.

Microscopy

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 Leica Microscope with the Q Capture Pro software (Surrey, BC, Canada) for imaging. Raw images were converted to solid contrasting colors using Open Lab software (Perkin Elmer, Waltham, MA) and area and feret diameter were calculated using the Image Pro Plus software (MediaCybernetics, Rockville, MD).

Statistical Analysis

All data were statistically analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC). Daily temperature indices and production data were analyzed by repeated measures, using PROC MIXED with an autoregressive covariance structure and week of the experiment as the repeated effect. The model included

treatment, week, and their interaction as fixed effects; and initial body weight was used as a covariate. Plasma metabolites and proteins were analyzed by repeated measures, using PROC MIXED with a spatial power law covariance structure and day of the experiment as the repeated effect. The model included treatment, day, and their interaction as fixed effects; and the initial measure for each blood parameter was used as a covariate. Moisture content/percentage, backfat thickness, and fatty acid composition/percentage of the adipose samples were analyzed using PROC MIXED with no covariate. Data are reported as least square means and considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

Results:

Experiment 1

No statistical interactions between dietary treatment and environmental conditions were detected (Table 2A-6A; $P > 0.05$) so the main effect of diet and treatments are reported. Decreased growth performance was caused by both HS and the PFTN treatments ($P < 0.01$). HS decreased feed intake ($P < 0.01$) and by experimental design PFTN had a similar feed intake to HS ($P < 0.01$). Pigs in the PFTN treatment had the most efficient conversion of BW gain, while HS pigs were least feed efficient ($P < 0.01$). Regardless of environment, increasing the level of dietary fat and the degree of unsaturation of the lipid source tended to increase feed efficiency ($P = 0.07$).

As expected HS pigs had increased respiration rates and rectal temperatures (Table 3A; $P < 0.001$), but neither thermal variable were influenced by dietary fat ($P \geq 0.65$).

Hot carcass weight and belly weight of the TN ad libitum carcasses were the heaviest (Table 4A, $P < 0.001$). Carcass yield trended to be decreased in the PFTN pigs ($P = 0.096$). Adding 3%CO decreased loin muscle depth ($P < 0.01$). Back fat was thickest in the TN ad-libitum fed pigs and thinnest in the PFTN ($P < 0.01$). Fat free lean percent tended to be lower in TN carcasses ($P = 0.09$). Including 3%CO increased belly weights ($P = 0.02$). Belly thickness of the TN bellies were greater when measured in the center ($P = 0.03$) and resulted in a trend when measured on the scribe edge ($P = 0.06$). Fat color was altered via l star being lower and a star being greater in TN bellies ($P \leq 0.02$). No other differences due to environmental conditions or dietary treatment were evident ($P > 0.10$).

Environmental conditions in did not influence fatty acid profiles or IV (Table 5A; $P \geq 0.055$). As expected increasing the degree of unsaturation of the dietary fat source increased linoleic acid and IV on d 21 and at market (d 35) ($P < 0.001$).

Inclusion of dietary fat, and a source that was unsaturated increased ATTD of AEE (Table 6A; $P < 0.001$). True total track digestibility of AEE in 3%CO-based diets was increased than that of CNTR and 3%TAL-based diets ($P = 0.012$). Environment had no impact of TTTD of AEE ($P = 0.118$).

Experiment 2

Vitals Measurements

During P2, RR was increased by 88% (48 bpm average increase) in all HS treatments relative to TNAL and TNPF pigs ($P < 0.01$; Figure 1). There was an overall increase (1.2°C) in T_R PM in all pigs exposed to HS conditions relative to the two TN treatments ($P < 0.01$; Table 2B and Figure 2). Interestingly, HSSO fed pigs had increased (0.41°C) T_R PM relative to the other two HS treatments during weeks 3 and 4 of the experiment ($P < 0.05$; Figure 2). Skin temperature was also increased in all HS treatments relative to TNAL and TNPF during P2 (data not shown).

Production Parameters

Compared to TNAL pigs during P2, HS decreased FI by 31% ($P < 0.01$) and 23% ($P < 0.01$) during the first and last two weeks, respectively (Figure 3). Body weight was decreased in all HS treatments by 4% ($P < 0.01$), 6% ($P < 0.01$), and 7% ($P < 0.01$) on d 8, 15, and 21 compared to TNAL fed pigs (Figure 4). ADG was also decreased similarly in all HS treatments by 70% ($P < 0.01$) and 36% ($P < 0.01$) during weeks 2 and 3 as compared to TNAL fed pigs (Figure 5). By experimental design, the TNPF pigs exhibited the same magnitude and pattern of FI reduction as compared to the HS pigs. TNPF and HS pigs had a similar ADG but ADG nor FI was influenced by SO or Cr supplementation (Table 3B and Figure 5). There was also no overall treatment effect on FE ($P = 0.12$; Table 3B and Figure 6).

Metabolic/Plasma Parameters.

Overall, TNAL fed pigs had increased plasma insulin relative to TNPF (0.15 vs. 0.10 µg/L, $P < 0.01$; Figure 7 & 8). HS also increased insulin levels compared to TNPF (0.12 vs. 0.10 µg/L, $P = 0.02$; Figure 7 and 8) and this increase was not influenced by SO or Cr supplementation. The insulin to FI ratio was only numerically increased in all HS treatments relative to TNPF ($P = 0.18$; Figure 9 & 10).

Post-Mortem Measurements

Fatty Acid Composition

In abdominal adipose, there was an overall decrease in C16:1 in all HS treatments compared to TNAL ($P = 0.06$; Table 4B) and TNPF ($P = 0.03$; Table 4B) pigs and this fatty acid was reduced further with SO supplementation ($P < 0.05$; Table 4B) relative to the other HS treatments. However, HSCr pigs did not differ from TNAL ($P = 0.69$; Table 4B) or TNPF ($P = 0.59$; Table 4B). HS increased C17:0 levels compared to TNAL ($P = 0.03$; Table 4B) and TNPF ($P = 0.05$; Table 4B). SO supplementation increased C18:0 content ($P < 0.01$; Table

4B) compared to all other treatments with no major differences detected in other fatty acid comparisons. C18:1 c9 was decreased in all HS treatments compared to TNAL ($P = 0.01$; Table 4B) and TNPF ($P = 0.05$; Table 4B) and this reduction was most pronounced with SO supplementation ($P < 0.01$; Table 4B). C18:1 c9 content did not differ between HSCr and TNAL ($P = 0.56$; Table 4B) and HSCr and TNPF ($P = 0.15$; Table 4B). There was an increase in C18:2 n6 in HS compared to TNAL ($P = 0.01$; Table 4B), but these levels did not differ from TNPF ($P = 0.15$; Table 4B). TNPF pigs had increased C20:0 compared to all HS treatments ($P < 0.01$; Table 4B) and TNAL ($P = 0.04$; Table 4B). SO supplementation decreased C20:1 content compared to all other treatments ($P < 0.05$; Table 4B). HS increased 18:3 n3 content compared to TNAL ($P = 0.01$; Table 4B), but did not differ relative to TNPF ($P = 0.17$; Table 4B).

In inner subcutaneous adipose, HS decreased C16:1 content compared to TNPF ($P = 0.03$; Table 5B) and it tended to be decrease compared to TNAL ($P = 0.07$; Table 5B). There was an increase in C17:0 for all HS treatments compared to TNAL ($P = 0.05$; Table 5B) and TNPF ($P = 0.02$; Table 5B). HS increased C18:0 levels compared to TNAL ($P = 0.05$; Table 5B). HSSO fed pigs had increased C18:0 relative to all other treatments ($P < 0.01$; Table 5B). C18:1 c9 was decreased in all HS treatments compared to TNAL ($P < 0.01$; Table 5B) and it tended to be reduced compared to TNPF fed pigs ($P = 0.06$; Table 5B). SO supplementation further reduced C18:1 c9 content relative to HSAL ($P < 0.01$; Table 5B) and HSCr ($P < 0.01$; Table 5B). HS decreased C20 compared to TNPF ($P = 0.03$; Table 5B), but it did not differ from TNAL ($P = 0.45$; Table 5B). C20 content did not differ between HSCr and TNAL ($P = 0.23$; Table 5B). C20:1 content for HSAL pigs did not differ from TNAL ($P = 0.40$; Table 5B) or TNPF ($P = 0.69$; Table 5B). Interestingly, C20:1 supplementation was reduced by SO supplementation ($P < 0.01$; Table 5B) and tended to be decreased by Cr supplementation ($P = 0.09$; Table 5B) relative to HSAL. TNPF pigs had reduced 20:3 n6 content compared to all treatments ($P < 0.05$; Table 5B).

In outer subcutaneous adipose, there was an overall decrease in C16:1 content in all HS treatments relative to TNAL ($P < 0.01$; Table 6B) and TNPF ($P = 0.02$; Table 6B). C17:0 exhibited an overall increase in all HS treatments relative to TNAL ($P = 0.02$; Table 6B) and TNPF ($P = 0.02$; Table 6B). HS increased C18:0 content relative to TNAL ($P = 0.01$; Table 6B) and TNPF ($P = 0.05$; Table 6B). There was an increase in C18:0 levels in HSSO compared to all other treatments ($P < 0.05$; Table 6B). HS decreased C18:1 c9 content relative to TNAL ($P < 0.01$; Table 6B). Interestingly, Cr supplmentation increased C18:1 c9 levels compared to HSAL ($P = 0.04$; Table 6B). SO supplementation decreased 20:1 content compared to all other treatments ($P < 0.01$; Table 6B). HS increased 18:3 n3 levels compared to TNAL ($P = 0.02$; Table 6B). Interestingly, TNPF also had increased 18:3 n3 content relative to TNAL ($P = 0.05$; Table 6B).

IV values were assessed to evaluate the overall degree of unsaturation at each adipose tissue depot. In general, there were no treatment differences in IV values in abdominal ($P = 0.40$; Table 4B), inner subcutaneous

(P = 0.13; Table 5B), or outer subcutaneous (P = 0.79; Table 6B) adipose depots. Feeding SO decreased the desaturase index at all adipose depots analyzed (P < 0.01; Tables 4B, 5B, and 6B).

Adipocyte Area

In abdominal adipose tissue, there was a decrease in adipocyte area in TNPF relative to TNAL (P = 0.01; Table 7B and Figure 11). Interestingly, SO and Cr supplementation decreased adipocyte area relative to HSAL (P = 0.01; Table 7B and Figure 11), but they did not differ compared to TNAL or TNPF (Table 7B and Figure 11). There were no differences in adipocyte area detected at inner and outer subcutaneous adipose depots.

Adipose Moisture Content

Regardless of SO and Cr supplementation, all HS treatments tended to increase moisture content at the abdominal (P = 0.09; Table 8B and Figure 12) and inner subcutaneous (P = 0.06; Table 8B and Figure 12) adipose depots relative to TNAL. Interestingly, TNPF pigs had increased moisture content at abdominal (P = 0.01; Table 8B and Figure 12) and outer subcutaneous (P = 0.01; Table 8B and Figure 12) adipose depots and tended to be increased in inner subcutaneous adipose tissue (P = 0.07; Table 8B and Figure 12) relative to TNAL. TNPF adipose moisture was also increased at the outer subcutaneous adipose depot relative to all HS treatments (P = 0.04; Table 8B and Figure 12).

Discussion

Despite aggressive heat abatement strategies, HS still represents a major economic burden to the U.S. swine industry with an estimated $900 million in annual losses during the warm summer months (Pollman 2010). Sources of reduced revenue include; slower growth rates, inefficient feed utilization, increased health care costs, inconsistent market weights, mortality, and altered carcass traits. The effect of heat stress will likely become an even more significant issue in the future if the frequency of severe hot weather increases as predicted.

In the first experiment, HS decdreased FI, resulting in decreased growth performance and carcass weight, but it did not appear to alter deposited fatty acid composition or carcass iodine value. Carcass iodine value was affected by dietary fat source, suggesting that fatty acid intake or concentration can be used as a predictor of carcass iodine value (Kellner et al., 2014). Importantly this response to dietary fat was not influence by HS. From a practical standpoint, traditional administration of dietary fat during summer months may not fully overcome the effects of heat stress.

In the second experiment, pigs allocated to the three HS treatments experienced a significant heat load. Interestingly, the HSSO group had higher 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 that SO supplementation was able

to attenuate the activity of the Δ^9 desaturase enzyme. Importantly, this enzyme has been implicated in thermoregulation as stearoyl CoA desaturase knockout mice demonstrate impaired thermoregulatory capacity and become hypothermic when reared in a 4°C environment (Lee et al., 2004). Disrupting the enzyme with dietary SO supplementation in this experiment had the opposite effect, as HSSO pigs demonstrated relatively increased hyperthermia during HS. Identifying whether or not the Δ^9 desaturase enzyme could be manipulated to help pigs maintain a healthy body temperature during HS is of further interest.

Accompanying the increases in body temperature indices, decreases in FI, BW, and ADG were observed in the three HS treatments. Although the HSSO pigs had incdreased T_R relative to the other two HS treatments during the last two weeks of P2, we did not observe a proportional reduction in FI and this is comparable to results found by our group Seibert et al. 2015. Interestingly, while HSAL and HSSO treatments exhibited a plateau effect from week 3 to 4 for ADG and FE, HSCr pigs displayed a positive slope for the two production parameters, which may be attributed to Cr's association with low molecular weight chromium-binding substance (LMWCr) and concomitant ability to potentiate insulin action (Davis and Vincent, 1997). Although evaluating the effect of our dietary supplements on production characteristics was not our primary objective, it would have been interesting to carry out the study for another week to see if the HSCr pigs maintained their upward trend in ADG and FE (Figures # and #).

Our primary objective in this experiment was to investigate the "flimsy fat" or "balloon syndrome" phenotype in response to HS, which negatively affects processing and final product value. The very name of the phenotype suggests an overall increase in FA unsaturation, which would confer loss of tissue firmness due to the presence of kinks in FA tails. Furthermore, the heat-induced elevation in circulating insulin stimulation should enhance the abundance and activity of the insulin-sensitive SCD enzyme, which should promote FA unsaturation (Dobrzyn et al., 2010). However, cell membranes become more fluid-like at higher temperatures and so increased formation of saturated FAs maintain the integrity of the plasma membrane, thus there should be an overall decrease in FA unsaturation during HS. In agreement with this homeoviscous adaptation concept, HS caused decreases in C16:1 (palmitoleic) and C18:1 c9 (oleic), but increased C17:0 (margaric) and C18:0 (stearic) in all adipose depots compared to TNAL. Heat-induced increases in saturated fatty acid content in pigs has also been observed elsewhere (Lefaucheur et al., 1991; Katsumata et al., 1995; Kouba et al., 1999). 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). Thus it appears that altered fatty acid profiles are unlikely contributing to the decreased carcass fat quality during heat stress.

We also evaluated adipocyte size because it has been previously reported to be associated with adipose tissue firmness (Mendizabal et al., 2004). We hypothesized that the heat-induced insulin response would promote an overall decrease in adipocyte size because of insulin's ability to stimulate adipocyte proliferation. Although

we observed increased circulating insulin levels in HS treatments in agreement with previous reports (Pearce et al., 2013; Sanz Fernandez et al., 2015), we surprisingly observed a numeric increase in adipocyte size in abdominal adipose tissue for HSAL compared to TNAL. Interestingly, TNPF, HSSO, and HSCr exhibited smaller adipocyte sizes relative to HSAL. Although previous studies have reported increases in adipocyte diameters in back fat of pigs (Rinaldo and Le Dividich, 1991) and decreases in epididymal fat of rats (Cherqui et al., 1979) reared in HS and cold conditions, respectively, perhaps we did not observe temperature induced differences in adipose size because of our experimental design (eg. constant vs diurnal heat pattern). No differences in adipocyte size were detected at inner or outer subcutaneous adipose tissues.

Interestingly, we observed an increase in moisture content in all HS treatments at all adipose depots relative to our TNAL control group. Interestingly, this was also observed in TNPF pigs, suggesting that the increase in adipose hydration may be linked to decreased feed intake and thus less adipocyte "filling". Interestingly obese humans on a low calorie diet have increased water content to abdominal adipose tissue (Laaksonen et al., 2003).

In summary, despite the occurrence of the "flimsy fat" phenotype in response to HS, the overall degree of FA unsaturation remained unchanged in HS pigs at abdominal, inner, and outer subcutaneous adipose depots. HS also did not significantly influence adipocyte size, but was affected in TNPF pigs. Interestingly, all HS treatments exhibited increased adipose moisture content at all three depots, but this may be indirectly linked to the reduction in FI. Whether or not the increased moisture content is linked to the altered carcass fat quality in response to HS is of scientific and practical interest, but will require a more detailed investigation.

Table 1A. Ingredient composition (as-fed basis) of the experimental diets formulated with 3% corn oil (CO), 3% tallow (TAL), or no added fat (control [CNTR])

	Treatment		
Item	CNTR	3% CO	3% TAL
Ingredient, %			
Corn	84.36	79.74	79.74
Soybean meal (46.5% CP)	12.71	14.35	14.35
CO	-	3.00	-
TAL	-	-	3.00
Limestone	0.90	0.90	0.90
Monocalcium phosphate	0.56	0.53	0.53
Salt	0.50	0.50	0.50
L-lysine HCL	0.15	0.15	0.15
DL-methionine	-	0.01	0.01
L-threonine	0.01	0.01	0.01
Vitamin premix[1]	0.20	0.20	0.20
Trace mineral premix[2]	0.15	0.15	0.15
Titanium dioxide	0.40	0.40	0.40
Santoquin[3]	0.06	0.06	0.06
Analyzed composition			
DM, %	88.65	89.01	88.39
GE, mcal/kg	3.81	4.01	3.95
Crude protein (N × 6.25), %	13.16	13.56	13.55
Crude fat, %	3.18	6.21	6.22
Dietary fat IV[4], g/100g	-	123.0	41.8
Diet IV[5], g/100g	117.9	120.8	84.6
Diet IVP[6]	37.5	75.0	52.6

[1]Provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.023 mg vitamin B12 per kg of diet.

[2]Provided 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kg of diet.

[3]Santoquin Mixture 6 (Feed and forage Anti-oxidant; NOVUS International, Saint Charles, MO).

[4]Iodine value (IV) determined via titration (Barrow-Agee Labs, Memphis, TN)

[5]Iodine value calculated from fatty acid composition: IV = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C22:1] × 0.723; brackets indicate concentration (AOCS, 1998).

[6]Iodine value product (IVP) = (IV of the dietary lipids) × (% dietary lipid) × 0.10 (Madsen et al., 1992).

Table 2A. Effects of ad-libitum feed intake in thermal neutral conditions (TN)[1], pair-feeding in thermal neutral conditions (PFTN)[1], or heat stress (HS)[2] and additional inclusion of no dietary fat (CNTR), 3% tallow (TAL), or 3% corn oil (CO) on growth performance

	Treatments										P value[3]			
	TN			PFTN			HS							
Parameter	CNTR	TAL	CO	CNTR	TAL	CO	CNTR	TAL	CO	SEM	T	E	DF	E × DF
ADG, kg	1.00[a]	0.99[a]	1.10[a]	0.78[b]	0.80[b]	0.74[b]	0.72[b]	0.69[b]	0.76[b]	0.04	<0.01	<0.01	0.49	0.41
ADFI, kg	3.58[a]	3.35[a]	3.44[a]	2.54[b]	2.42[b]	2.52[b]	2.54[b]	2.41[b]	2.51[b]	0.13	<0.01	<0.01	0.12	0.98
G:F, kg	0.287[bc]	0.295[bc]	0.320[ab]	0.307[abc]	0.332[a]	0.317[ab]	0.282[c]	0.284[c]	0.304[abc]	0.016	0.01	< 0.01	0.07	0.50

[a-c]Within a row, least squares means lacking a common superscript differ, $P < 0.05$.

[1]Constant thermal neutral environment of ~24.0°C.

[2]Diunral heat stress environment of ~33.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h from d 0 to d 7, ~33.5°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 7 to d 14, ~34.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 14 to d 21, ~34.5°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 21 to d 28, and ~35.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 28 to d 35.

[3]Probability values for main effects of treatment (T), environment (E), and dietary fat (DF), as well as the environment × dietary fat interaction (E × DF).

Table 3A. Effects of ad-libitum feed intake in thermal neutral conditions (TN)[1], pair-feeding in thermal neutral conditions (PFTN)[1], or heat stress (HS)[2] and additional inclusion of no dietary fat (CNTR), 3% tallow (TAL), or 3% corn oil (CO) on daily rectal temperature and respiration rate[3]

	Treatments										P value[4]			
	TN			PFTN			HS							
Parameter	CNTR	TAL	CO	CNTR	TAL	CO	CNTR	TAL	CO	SEM	T	E	DF	E × DF
RR[5], bpm	37.1[b]	36.8[b]	35.0[b]	34.8[b]	34.5[b]	33.8[b]	79.3[a]	77.4[a]	78.1[a]	2.1	<0.01	<0.01	0.69	0.90
RT[6], °C	38.1[b]	38.1[b]	38.2[b]	38.2[b]	38.2[b]	38.2[b]	39.0[a]	39.0[a]	38.9[a]	0.1	<0.01	<0.01	0.65	0.19

[a-c]Within a row, least squares means lacking a common superscript differ, $P < 0.05$.

[1]Constant thermal neutral environment of ~24.0°C.

[2]Diunral heat stress environment of ~33.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h from d 0 to d 7, ~33.5°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 7 to d 14, ~34.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 14 to d 21, ~34.5°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 21 to d 28, and ~35.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 28 to d 35.

[3]Measured daily at 1100h

[4]Probability values for main effects of treatment (T), environment (E), and dietary fat (DF), as well as the environment × dietary fat interaction (E × DF).

[5]Respiration rate (breaths per minute)

[6]Rectal temperature (°C)

Table 4A. Effects of ad-libitum feed intake in thermal neutral conditions (TN)[1], pair-feeding in thermal neutral conditions (PFTN)[1], or heat stress (HS)[2] and additional inclusion of no dietary fat (CNTR), 3% tallow (TAL), or 3% corn oil (CO) on carcass characteristics, loin characteristics, and belly characteristics

	Treatments										P value[3]			
	TN			PFTN			HS							
Parameter	CNTR	TAL	CO	CNTR	TAL	CO	CNTR	TAL	CO	SEM	T	E	DF	E × DF
HCW, kg	100.6^{a}	100.4^{a}	103.6^{a}	92.5^{b}	93.5^{b}	93.3^{b}	92.5^{b}	91.3^{b}	92.5^{b}	1.8	<0.001	<0.001	0.554	0.827
Yield, %	74.0	74.4	74.0	72.5	73.7	73.3	74.0	73.9	73.4	0.6	0.330	0.096	0.407	0.600
LM depth, cm	6.50	6.35	5.84	5.96	6.40	5.50	5.87	6.18	5.86	0.30	0.059	0.261	0.006	0.387
Back fat, cm	2.33	2.23	2.31	2.01	1.97	1.99	2.23	1.98	2.10	0.23	0.090	0.011	0.353	0.854
Fat free lean, %	52.5	52.9	51.8	53.8	54.5	53.3	52.3	54.2	53.1	1.6	0.146	0.088	0.129	0.774
Loin ultimate pH	5.63	5.67	5.65	5.64	5.66	5.63	5.65	5.65	5.65	0.05	0.752	0.873	0.199	0.639
LCS[4]	3.0	3.4	3.1	2.9	3.0	2.9	3.0	2.9	3.2	0.2	0.859	0.560	0.806	0.693
LMS[5]	1.8	1.8	1.8	1.6	1.9	1.7	1.7	1.6	1.7	0.2	0.778	0.495	0.829	0.515
Belly weight, kg	8.54^{ab}	8.02^{ab}	9.21^{a}	7.71^{c}	7.73^{c}	7.98^{bc}	7.63^{c}	7.28^{c}	7.65^{c}	0.29	<0.001	<0.001	0.018	0.372
Belly ET[5], cm	3.24	2.83	3.25	2.85	3.00	2.59	2.74	2.76	2.80	0.29	0.212	0.055	0.855	0.313
Belly MT[6], cm	2.39	2.46	2.56	2.20	2.20	2.29	2.23	2.11	2.24	0.14	0.297	0.028	0.568	0.919
l star	72.5^{abc}	72.3^{bc}	70.8^{c}	74.2^{a}	72.7^{abc}	73.4^{ab}	73.5^{ab}	72.3^{bc}	73.6^{ab}	0.8	0.037	0.022	0.177	0.309
a star	11.0^{abc}	11.4^{ab}	12.4^{a}	9.5^{c}	10.2^{bc}	9.9^{bc}	10.3^{bc}	10.9^{abc}	9.8^{c}	0.6	0.020	0.003	0.452	0.318
b star	7.3	7.6	8.0	7.1	7.2	7.7	7.4	7.7	7.3	0.3	0.247	0.303	0.210	0.215
Durometer	45.5	45.3	42.5	41.8	40.0	43.8	46.7	40.1	41.4	3.6	0.705	0.682	0.547	0.687
Belly firmness[7]	2.3	1.9	2.4	2.3	2.5	2.6	2.4	2.3	2.6	0.2	0.406	0.243	0.220	0.720

[a-c]Within a row, least squares means lacking a common superscript differ, $P < 0.05$.

[1]Constant thermal neutral environment of ~24.0°C.

[2]Diunral heat stress environment of ~33.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h from d 0 to d 7, ~33.5°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 7 to d 14, ~34.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 14 to d 21, ~34.5°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 21 to d 28, and ~35.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 28 to d 35.

[3]Probability values for main effects of treatment (T), environment (E), and dietary fat (DF), as well as the environment × dietary fat interaction (E × DF).

4Loin Color Score; evaluated postmortem according to the Japanese color bar 1 to 6 scale, 1 = extremely light, 6 = extremely dark (Sullivan et al., 2007).

5Loin Marbling Score; evaluated postmortem according to National Pork Board Standards (NPPC, 2000). The marbling standards correspond to percentage of intramuscular lipid.

[6]Measured in the middle scribe side of the belly.

[7]Measured in the middle of the belly.

[8]Measured by a subjective score of 1, 2, or 3 with 1 being the firmest.

Table 5A. Effects of ad-libitum feed intake in thermal neutral conditions (TN)[1], pair-feeding in thermal neutral conditions (PFTN)[1], or heat stress (HS)[2] and additional inclusion of no dietary fat (CNTR), 3% tallow (TAL), or 3% corn oil (CO) on deposited fatty acid concentration and iodine value (IV)[3]

	Treatments										P value[4]			
	TN			PFTN			HS							
Parameter	CNTR	TAL	CO	CNTR	TAL	CO	CNTR	TAL	CO	SEM	T	E	DF	E × DF
d 7														
14:0, %	1.11	1.05	1.14	1.10	1.02	1.04	1.11	1.11	1.13	0.04	0.191	0.055	0.201	0.557
16:0, %	22.27	22.33	22.51	22.61	21.62	21.88	22.37	22.34	22.37	0.35	0.686	0.440	0.525	0.566
16:1, %	2.67	2.34	2.31	2.25	2.17	2.23	2.47	2.17	2.32	0.18	0.358	0.270	0.166	0.848
18:0, %	10.65	10.64	11.19	11.35	11.13	11.22	10.94	11.69	10.96	0.48	0.627	0.461	0.861	0.475
18:1, %	45.38^{a}	44.81^{ab}	42.88^{c}	43.79^{abc}	45.34^{a}	44.68^{abc}	44.33^{abc}	43.33^{bc}	43.32^{bc}	0.62	0.026	0.101	0.140	0.062
18:2, %	13.91	14.70	15.79	14.91	14.66	15.01	14.71	15.17	15.86	0.60	0.331	0.527	0.093	0.752
18:3, %	0.62	0.62	0.66	0.64	0.64	0.65	0.66	0.68	0.69	0.04	0.580	0.117	0.556	0.957
20:1, %	0.89	0.99	0.92	0.96	0.92	0.88	0.93	0.90	0.86	0.06	0.485	0.468	0.305	0.497
IV, g/100 g	68.22	68.81	69.08	68.29	69.06	69.17	68.61	68.30	69.62	0.87	0.917	0.976	0.425	0.929
d 21														
14:0, %	1.18	1.13	1.09	1.06	1.05	1.10	1.12	1.14	1.12	0.04	0.399	0.109	0.785	0.454
16:0, %	22.71	22.24	21.71	21.86	21.75	21.80	21.99	22.02	21.69	0.35	0.639	0.370	0.294	0.768
16:1, %	2.65	2.72	2.34	2.33	2.37	2.58	2.42	2.43	2.54	0.17	0.701	0.574	0.951	0.382
18:0, %	10.56	10.30	10.44	10.83	10.71	9.92	10.52	10.73	10.01	0.47	0.524	0.970	0.162	0.662
18:1, %	45.70	47.41	44.62	45.60	47.07	45.52	45.88	45.32	44.88	0.75	0.107	0.349	0.022	0.251
18:2, %	13.44^{bcd}	12.20^{d}	15.69^{a}	14.15^{abc}	13.20^{cd}	15.37^{ab}	14.09^{abcd}	14.20^{abc}	15.65^{a}	0.63	0.001	0.197	<0.001	0.473
18:3, %	0.59	0.53	0.61	0.62	0.57	0.62	0.62	0.62	0.66	0.04	0.287	0.125	0.124	0.818
20:1, %	0.89	1.08	1.01	1.08	1.01	0.89	0.97	0.95	0.95	0.27	0.251	0.697	0.340	0.094
IV, g/100 g	67.55^{d}	66.93^{d}	70.40^{abc}	68.60^{bcd}	68.11^{cd}	70.77^{ab}	68.72^{bcd}	68.46^{cd}	70.78^{a}	0.94	0.004	0.259	<0.001	0.960
d 35														
14:0, %	1.12	1.10	1.10	1.05	1.05	1.02	1.06	1.10	1.08	0.04	0.902	0.257	0.902	0.955
16:0, %	21.72	21.88	22.05	21.64	21.65	20.79	21.80	21.81	21.57	0.34	0.569	0.211	0.508	0.576
16:1, %	2.52	2.44	2.21	2.38	2.17	2.16	2.35	2.33	2.41	0.53	0.572	0.338	0.327	0.477
18:0, %	10.12	10.59	10.81	10.47	10.93	9.90	11.01	10.93	10.16	0.49	0.114	0.529	0.162	0.138
18:1, %	47.18^{ab}	46.84^{ab}	43.61^{b}	47.83^{a}	47.21^{ab}	44.73^{ab}	46.39^{ab}	45.97^{ab}	45.61^{b}	0.77	0.010	0.497	<0.001	0.178
18:2, %	13.63^{d}	13.29^{d}	16.27^{ab}	12.82^{d}	13.15^{d}	17.26a	13.67^{d}	13.87^{cd}	15.35^{bc}	0.62	<0.001	0.961	<0.001	0.116
18:3, %	0.61	0.61	0.66	0.58	0.61	0.72	0.62	0.64	0.67	0.03	0.083	0.707	0.003	0.533
20:1, %	0.92	0.96	0.93	1.05	0.96	0.87	0.93	0.93	0.95	0.05	0.492	0.767	0.351	0.245
IV, g/100 g	69.11^{bcd}	68.20^{d}	70.50^{bc}	68.13^{d}	67.97^{d}	73.20^{a}	68.34^{d}	68.43^{cd}	70.82^{b}	0.98	<0.001	0.624	<0.001	0.199

[a-c]Within a row, least squares means lacking a common superscript differ, $P < 0.05$.

[1]Constant thermal neutral environment of ~24.0°C.

[2]Diunral heat stress environment of ~33.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h from d 0 to d 7, ~33.5°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 7 to d 14, ~34.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 14 to d 21, ~34.5°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 21 to d 28, and ~35.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 28 to d 35.

[3]Measured at the jowl

[4]Probability values for main effects of treatment (T), environment (E), and dietary fat (DF), as well as the environment × dietary fat interaction (E × DF).

Table 6A. Effects of ad-libitum feed intake in thermal neutral conditions (TN)[1], pair-feeding in thermal neutral conditions (PFTN)[1], or heat stress (HS)[2] and additional inclusion of no dietary fat (CNTR), 3% tallow (TAL), or 3% corn oil (CO) on apparent total track digestibility (ATTD) and true total track digestibility (TTTD) of acid hydrolyzed ether extract (AEE) on d 17

	Treatments										P value[4]			
	TN			PFTN			HS							
Parameter	CNTR	TAL	CO	CNTR	TAL	CO	CNTR	TAL	CO	SEM	T	E	DF	E × DF
ATTD, %	41.3^{e}	67.9^{bcd}	71.5^{ab}	42.9^{de}	68.4^{bcd}	72.8^{a}	40.4^{e}	67.3^{cd}	69.4^{bc}	1.3	<0.001	0.054	<0.001	0.886
TTTD[5], %	97.1	96.4	100.1	98.8	96.8	99.9	96.2	95.7	98.0	0.7	0.082	0.118	0.012	0.932

[a-c]Within a row, least squares means lacking a common superscript differ, $P < 0.05$.

[1]Constant thermal neutral environment of ~24.0°C.

[2]Diunral heat stress environment of ~33.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h from d 0 to d 7, ~33.5°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 7 to d 14, ~34.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 14 to d 21, ~34.5°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 21 to d 28, and ~35.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 28 to d 35.

[3]Measured daily at 1100h

[4]Probability values for main effects of treatment (T), environment (E), and dietary fat (DF), as well as the environment × dietary fat interaction (E × DF).

[5]TTTD (%) of AEE was calculated by correcting ATTD of AEE for endogenous fat losses at 20 g of AEE/kg of dry matter intake.

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

Ingredients	---- % ----
Corn	73.77
Soybean meal	9.36
Dried distillers grains	15.0
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
Crude fiber	2.75
Ash	3.75

[1]0.97% Limestone, 0.37% Salt, 0.11% Vitamin and Trace Mineral (Provided 441,145 IU vitamin A, 80,877 IU vitamin D, 2,353 IU vitamin E, 118 IU vitamin K, 1,119 mg niacin, 884 mg pantothenic acid, 222 mg riboflavin, 269 µg choline, 22 µg folic acid, 1,473 µg vitamin B12, 79 µg biotin, 5,879 PPM zinc, 2,818 PPM manganese, 7,650 PPM iron, 1,072 PPM copper, 40 PPM iodine, 13 PPM selenium per kg of diet), 0.02% Rono M 10,000

Table 2B. Effects of treatments on body temperature indices.

	Treatments						*P*					
Parameter	TNAL[1]	TNPF[2]	HSAL[3]	HSSO[4]	HSCr[5]	SEM	Trt[6]	Wk[7]	Trt*Wk[8]	TNAL vs. HS[9]	TNPF vs. HS	TNAL vs. TNPF
Tr AM[10], °C	39.63	39.62	39.7	39.71	39.66	0.07	0.84	<0.01	0.09	0.40	0.42	0.98
Ts AM[11], °C	31.80[a]	30.63[b]	33.25[c]	33.41[c]	33.22[c]	0.40	<0.01	<0.01	<0.01	<0.01	<0.01	0.04
RR AM[12], bpm	57.34[a]	51.75[a]	72.11[b]	72.41[b]	71.85[b]	2.76	<0.01	<0.01	<0.01	<0.01	<0.01	0.05
Tr PM[13], °C	39.87[a]	39.80[a]	40.68[b]	40.91[b]	40.65[b]	0.11	<0.01	<0.01	<0.01	<0.01	<0.01	0.48
Ts PM[14], °C	33.70[a]	32.76[b]	36.20[c]	36.22[c]	36.02[c]	0.21	<0.01	<0.01	<0.01	<0.01	<0.01	0.02
RR PM[15], bpm	60.75[a]	54.60[a]	92.40[b]	98.01[b]	93.62[b]	2.95	<0.01	<0.01	<0.01	<0.01	<0.01	0.07

[1]Thermalneutral *ad libitum*
[2]Thermalneutral pair-fed
[3]Heat stress *ad libitum*
[4]Heat stress sterculic oil
[5]Heat stress chromium
[6]Treatment
[7]Week
[8]Treatment by week interaction
[9]All heat stress treatments
[10]Rectal temperature at 0600
[11]Skin temperature at 0600
[12]Respiration rate in beats per minute at 0600
[13]Rectal temperature at 1800
[14]Skin temperature at 1800
[15]Respiration rate in beats per minute at 1800

Table 3B. Effects of treatments on production parameters.

	Treatments						*P*					
Parameter	TNAL[1]	TNPF[2]	HSAL[3]	HSSO[4]	HSCr[5]	SEM	Trt[6]	Wk[7]	Trt*Wk[8]	TNAL vs. HS[9]	TNPF vs. HS	TNAL vs. TNPF
FI[10]	3.26[a]	2.65[b]	2.44[b]	2.51[b]	2.33[b]	0.13	<0.01	<0.01	<0.01	<0.01	0.16	0.01
FBW[11]	128.6[a]	125.0[b]	123.7[b]	123.7[b]	123.7[b]	1.06	0.02	<0.01	<0.01	<0.01	0.33	0.03
ADG[12]	1.09[a]	0.71[b]	0.74[b]	0.72[b]	0.76[b]	0.05	<0.01	<0.01	0.02	<0.01	0.63	<0.01
FE[13]	0.33	0.26	0.29	0.27	0.32	0.02	0.12	<0.01	0.07	0.18	0.23	<0.01

[1]Thermalneutral *ad libitum*
[2]Thermalneutral pair-fed
[3]Heat stress *ad libitum*
[4]Heat stress sterculic oil
[5]Heat stress chromium
[6]Treatment
[7]Week
[8]Treatment by week interaction
[9]All heat stress treatments
[10]Feed intake (kg/d)
[11]Final body weight (kg)
[12]Average daily gain
[13]Feed efficiency

Table 4B. Effects of treatments on fatty acid profile of abdominal adipose tissue

	Treatments						*P*			
FA	TNAL[1]	TNPF[2]	HSAL[3]	HSSO[4]	HSCr[5]	SEM	Trt[6]	TNAL vs. HS[7]	TNPF vs. HS	TNAL vs. TNPF
12:00	0.07	0.07	0.08	0.08	0.07	0.01	0.25	0.13	0.20	0.83
14:00	1.28	1.28	1.40	1.40	1.46	0.07	0.29	0.11	0.09	0.97
15:00	0.03	0.03	0.05	0.05	0.03	0.01	0.16	0.19	0.17	0.90
16:00	25.86	25.46	25.92	25.78	26.36	0.61	0.88	0.82	0.43	0.54
16:01	1.51^{c}	1.53^{c}	1.30^{b}	1.02^{a}	1.46^{bc}	0.09	<0.01	0.06	0.03	0.90
17:00	0.24^{a}	0.25^{a}	0.34^{b}	0.32^{b}	0.29^{ab}	0.02	0.03	0.03	0.05	0.80
17:01	0.13	0.16	0.16	0.14	0.16	0.02	0.67	0.34	0.68	0.12
18:00	19.00^{a}	19.31^{a}	19.86^{a}	24.40^{b}	19.01^{a}	0.89	<0.01	0.17	0.24	0.68
18:1 c9	35.72^{c}	34.38^{bc}	31.98^{b}	28.10^{a}	33.49^{bc}	1.07	<0.01	0.01	0.05	0.20
18:2 n6	12.06^{a}	13.18^{ab}	14.83^{b}	14.63^{b}	13.99^{b}	0.74	0.07	0.01	0.15	0.10
20:00	0.28^{b}	0.31^{c}	0.26^{b}	0.28^{b}	0.23^{a}	0.01	<0.01	0.25	<0.01	0.04
20:01	0.62^{b}	0.58^{b}	0.58^{b}	0.47^{a}	0.56^{b}	0.03	0.05	0.05	0.27	0.45
18:3 n3	0.44^{a}	0.48^{ab}	0.54^{b}	0.55^{b}	0.52^{b}	0.03	0.10	0.01	0.17	0.14
20:2 n6	0.50	0.50	0.53	0.53	0.49	0.03	0.67	0.25	0.60	0.58
20:3 n6	0.06	0.05	0.07	0.08	0.06	0.01	0.43	0.49	0.25	0.12
22:01	0.20	0.24	0.27	0.25	0.25	0.02	0.15	0.02	0.53	0.04
Unidentified[8]	2.17^{b}	2.61^{c}	2.02^{ab}	1.99^{ab}	1.66^{a}	0.16	<0.01	0.11	<0.01	0.06
IV[9]	54.8	55.52	56.45	52.45	56.4	1.57	0.40	0.84	0.88	0.58
16:1/16:0	0.06^{bc}	0.06^{c}	0.05^{ab}	0.04^{a}	0.06^{bc}	<0.01	<0.01	0.07	0.02	0.71
18:1/18:0	1.90^{b}	1.79^{b}	1.63^{b}	1.26^{a}	1.77^{b}	0.12	<0.01	0.06	0.17	0.33
Δ^9 DI (16)[10]	0.06^{bc}	0.06^{c}	0.05^{b}	0.04^{a}	0.05^{bc}	<0.01	<0.01	0.07	0.02	0.70
Δ^9 DI (18)[11]	0.65^{b}	0.64^{b}	0.62^{b}	0.54^{a}	0.64^{b}	0.02	<0.01	0.05	0.11	0.39
Δ^9 DI (14,16,18)[12]	0.45^{b}	0.44^{b}	0.41^{b}	0.36^{a}	0.43^{b}	0.01	<0.01	0.04	0.08	0.49
Chain length <16	1.35	1.35	1.50	1.49	1.49	0.08	0.41	0.13	0.11	0.99
Chain length >16	96.48^{b}	96.04^{a}	96.48^{bc}	96.52^{bc}	96.85^{c}	0.16	0.02	0.40	<0.01	0.10
SFA[13]	46.66^{a}	46.54^{a}	47.79^{a}	52.26^{b}	47.36^{a}	1.40	0.05	0.22	0.19	0.92
MUFA[14]	38.14^{c}	36.79^{bc}	34.23^{b}	29.97^{a}	35.91^{bc}	1.14	<0.01	0.01	0.05	0.21
PUFA[15]	13.03^{a}	14.06^{ab}	15.96^{c}	15.79^{bc}	15.06^{bc}	0.80	0.07	0.01	0.12	0.17
Omega-3 FA	0.44	0.48	0.54	0.55	0.52	0.03	0.12	0.02	0.17	0.17
Omega-6 FA	12.59^{a}	13.64^{ab}	15.41^{b}	15.24^{b}	14.55^{b}	0.76	0.07	0.01	0.13	0.14
PUFA/SFA[16]	0.28	0.30	0.33	0.32	0.32	0.02	0.58	0.14	0.49	0.27
Omega-3/omega-6	0.04	0.04	0.04	0.04	0.04	<0.01	0.87	0.84	0.36	0.62
MUFA/SFA[17]	0.82^{b}	0.79^{b}	0.72^{b}	0.61^{a}	0.76^{b}	0.05	0.03	0.05	0.12	0.46
UFA/SFA[18]	1.10	1.10	1.05	0.92	1.08	0.06	0.30	0.33	0.36	0.91
IA[19]	0.61	0.60	0.63	0.71	0.63	0.03	0.17	0.24	0.20	0.87

[1] Thermoneutral *ad libitium*
[2] Thermoneutral pair-fed
[3] Heat stress *ad libitium*
[4] Heat stress sterculic oil
[5] Heat stress chromium
[6] Treatment
[7] All heat stress treatments
[8] Total unidentified peaks
[9] Iodine value
[10] Δ^9 desaturase index (C16:0)
[11] Δ^9 desaturase index (C18:0)
[12] Total Δ^9 desaturase index
[13] Total saturated fatty acids
[14] Total monounsaturated fatty acids
[15] Total polyunsaturated fatty acids
[16] Polyunsaturated to saturated fatty acid ratio
[17] Monounsaturated to saturated fatty acid ratio
[18] Unsaturated to saturated fatty acid ratio
[19] Index of Atherogenicity

Table 5B. Effect of treatments on fatty acid profile of inner subcutaneous adipose tissue

	Treatments						*P*			
FA	TNAL[1]	TNPF[2]	HSAL[3]	HSSO[4]	HSCr[5]	SEM	Trt[6]	TNAL vs. HS[7]	TNPF vs. HS	TNAL vs. TNPF
12:00	0.05	0.06	0.06	0.07	0.06	0.01	0.15	0.36	0.49	0.40
14:00	1.04	1.11	1.08	1.18	1.20	0.06	0.26	0.12	0.48	0.30
15:00	0.05	0.05	0.05	0.05	0.06	<0.01	0.12	0.54	0.04	0.55
16:00	21.23	21.27	21.29	22.33	21.88	0.56	0.56	0.40	0.38	0.95
16:01	1.63^{bc}	1.73^{c}	1.40^{ab}	1.27^{a}	1.51^{abc}	0.12	0.09	0.07	0.03	0.63
17:00	0.33^{ab}	0.33^{a}	0.41^{c}	0.40^{c}	0.39^{bc}	0.03	0.09	0.05	0.02	0.89
17:01	0.25	0.26	0.27	0.22	0.25	0.02	0.40	0.79	0.55	0.79
18:00	12.59^{a}	13.00^{a}	14.32^{a}	17.45^{b}	13.20^{a}	0.83	<0.01	0.05	0.10	0.64
18:1 c9	39.57^{c}	37.76^{b}	36.49^{b}	33.03^{a}	37.41^{b}	0.73	<0.01	<0.01	0.06	0.01
18:2 n6	17.53	17.91	18.74	18.51	19.09	0.68	0.50	0.15	0.27	0.60
20:00	0.19^{ab}	0.23^{d}	0.22^{cd}	0.21^{bc}	0.17^{a}	0.01	<0.01	0.45	0.03	<0.01
20:01	0.78^{c}	0.73^{bc}	0.75^{c}	0.59^{a}	0.67^{ab}	0.03	<0.01	0.01	0.18	0.13
18:3 n3	0.72	0.75	0.78	0.76	0.76	0.03	0.74	0.22	0.71	0.28
20:2 n6	0.82	0.82	0.84	0.77	0.83	0.04	0.71	0.92	0.87	0.95
20:3 n6	0.14^{b}	0.11^{a}	0.15^{b}	0.13^{b}	0.15^{b}	0.01	0.01	0.75	<0.01	<0.01
22:01	0.29	0.32	0.34	0.32	0.32	0.02	0.78	0.26	0.82	0.17
Unidentified[8]	2.96^{b}	3.63^{c}	2.82^{b}	2.75^{b}	2.14^{a}	0.22	<0.01	0.14	<0.01	0.09
IV[9]	68.62	67.90	68.03	64.36	69.40	1.40	0.13	0.48	0.72	0.59
16:1/16:0	0.08^{bc}	0.08^{c}	0.07^{ab}	0.06^{a}	0.07^{abc}	0.01	0.03	0.02	0.01	0.64
18:1/18:0	3.20^{c}	2.97^{bc}	2.62^{b}	1.98^{a}	2.85^{bc}	0.17	<0.01	<0.01	0.05	0.41
Δ^9 DI (16)[10]	0.07^{bc}	0.08^{c}	0.06^{ab}	0.05^{a}	0.07^{abc}	<0.01	0.02	0.02	0.01	0.65
Δ^9 DI (18)[11]	0.76^{c}	0.74^{bc}	0.72^{b}	0.66^{a}	0.74^{bc}	0.01	<0.01	0.02	0.08	0.32
Δ^9 DI (14,16,18)[12]	0.54^{c}	0.53^{bc}	0.51^{b}	0.46^{a}	0.52^{bc}	0.01	<0.01	0.01	0.06	0.24
Chain length <16	1.09	1.19	1.19	1.26	1.24	0.06	0.29	0.06	0.54	0.12
Chain length >16	95.95^{b}	95.18^{a}	95.99^{b}	95.99^{b}	96.62^{c}	0.23	<0.01	0.35	<0.01	0.06
SFA[13]	35.37^{a}	36.00^{a}	37.43^{a}	41.65^{b}	36.89^{a}	1.22	<0.01	0.06	0.11	0.62
MUFA[14]	42.47^{c}	40.79^{bc}	39.24^{b}	35.43^{a}	40.16^{b}	0.82	<0.01	<0.01	0.05	0.04
PUFA[15]	19.21	19.58	20.51	20.17	20.82	0.75	0.55	0.17	0.29	0.64
Omega-3 FA	0.74	0.75	0.78	0.76	0.76	0.03	0.94	0.49	0.71	0.71
Omega-6 FA	18.47	18.83	19.73	19.41	20.06	0.72	0.53	0.16	0.28	0.64
PUFA/SFA[16]	0.55	0.54	0.55	0.50	0.57	0.03	0.62	0.77	0.86	0.89
Omega-3/omega-6	0.04	0.04	0.04	0.04	0.04	<0.01	0.33	0.23	0.10	0.97
MUFA/SFA[17]	1.21^{c}	1.14^{bc}	1.06^{b}	0.87^{a}	1.09^{b}	0.04	<0.01	<0.01	0.04	0.24
UFA/SFA[18]	1.76^{b}	1.68^{b}	1.61^{b}	1.37^{a}	1.66^{b}	0.07	<0.01	0.03	0.12	0.41
IA[19]	0.41	0.43	0.43	0.50	0.44	0.02	0.12	0.19	0.33	0.51

[1] Thermoneutral *ad libitium*
[2] Thermoneutral pair-fed
[3] Heat stress *ad libitium*
[4] Heat stress sterculic oil
[5] Heat stress chromium
[6] Treatment
[7] All heat stress treatments
[8] Total unidentified peaks
[9] Iodine value
[10] Δ^9 desaturase index (C16:0)
[11] Δ^9 desaturase index (C18:0)
[12] Total Δ^9 desaturase index
[13] Total saturated fatty acids
[14] Total monounsaturated fatty acids
[15] Total polyunsaturated fatty acids
[16] Polyunsaturated to saturated fatty acid ratio
[17] Monounsaturated to saturated fatty acid ratio
[18] Unsaturated to saturated fatty acid ratio
[19] Index of Atherogenicity

Table 6B. Effects of treatments on fatty acid profile of outer subcutaneous adipose tissue

	Treatments						*P*			
FA	TNAL[1]	TNPF[2]	HSAL[3]	HSSO[4]	HSCr[5]	SEM	Trt[6]	TNAL vs. HS[7]	TNPF vs. HS	TNAL vs. TNPF
12:00	0.06	0.06	0.05	0.05	0.05	0.01	0.59	0.48	0.13	0.76
14:00	1.07	1.14	1.09	1.12	1.03	0.06	0.65	0.85	0.36	0.28
15:00	0.06	0.05	0.06	0.06	0.05	0.01	0.60	0.89	0.39	0.55
16:00	20.83	20.76	20.41	20.85	19.89	0.43	0.48	0.42	0.41	0.91
16:01	1.99^{b}	1.97^{b}	1.64^{a}	1.57^{a}	1.63^{a}	0.12	0.04	<0.01	0.02	0.89
17:00	0.33^{a}	0.34^{a}	0.42^{b}	0.42^{b}	0.39^{ab}	0.02	0.02	0.02	0.02	0.70
17:01	0.28	0.30	0.31	0.29	0.29	0.02	0.96	0.67	0.79	0.58
18:00	10.68^{a}	11.17^{ab}	12.14^{b}	13.83^{c}	11.80^{ab}	0.56	<0.01	0.01	0.05	0.58
18:1 c9	40.64^{d}	38.32^{bc}	37.87^{b}	35.72^{a}	39.43^{cd}	0.51	<0.01	<0.01	0.40	<0.01
18:2 n6	18.22	18.91	19.83	20.16	19.20	0.59	0.18	0.05	0.23	0.36
20:00	0.17	0.20	0.19	0.15	0.16	0.02	0.51	0.76	0.19	0.41
20:01	0.74^{b}	0.76^{b}	0.75^{b}	0.62^{a}	0.75^{b}	0.03	0.02	0.38	0.24	0.75
18:3 n3	0.73^{a}	0.79^{b}	0.83^{b}	0.82^{b}	0.78^{ab}	0.03	0.06	0.02	0.56	0.05
20:2 n6	0.83	0.85	0.89	0.81	0.90	0.03	0.21	0.40	0.61	0.75
20:3 n6	0.14	0.12	0.15	0.13	0.14	0.01	0.18	0.82	0.02	0.15
22:01	0.33	0.35	0.39	0.35	0.35	0.02	0.39	0.22	0.40	0.61
Unidentified[8]	3.13^{a}	3.91^{b}	3.03^{a}	3.06^{a}	3.21^{a}	0.24	0.08	0.90	<0.01	0.05
IV[9]	71.10	70.49	71.51	70.01	71.60	1.05	0.79	0.96	0.62	0.70
16:1/16:0	0.10^{c}	0.10^{bc}	0.08^{ab}	0.08^{a}	0.08^{ab}	0.01	0.05	<0.01	0.02	0.85
18:1/18:0	3.93^{c}	3.51^{bc}	3.18^{b}	2.61^{a}	3.37^{b}	0.20	<0.01	<0.01	0.04	0.27
Δ^9 DI (16)[10]	0.09^{c}	0.09^{bc}	0.07^{a}	0.07^{a}	0.08^{ab}	0.01	0.05	<0.01	0.02	0.84
Δ^9 DI (18)[11]	0.79^{c}	0.78^{bc}	0.78^{b}	0.72^{a}	0.77^{b}	0.01	<0.01	<0.01	0.05	0.26
Δ^9 DI (14,16,18)[12]	0.57^{c}	0.55^{bc}	0.54^{b}	0.51^{a}	0.56^{bc}	0.01	<0.01	0.01	0.18	0.21
Chain length <16	1.10	1.24	1.17	1.22	1.09	0.06	0.32	0.46	0.23	0.09
Chain length >16	95.77^{b}	94.84^{a}	95.80^{b}	95.72^{b}	95.70^{b}	0.24	0.04	0.93	<0.01	0.02
SFA[13]	33.05^{a}	33.72^{a}	34.32^{a}	36.47^{b}	33.32^{a}	0.78	0.03	0.11	0.26	0.63
MUFA[14]	43.92^{d}	41.70^{bc}	40.95^{b}	38.55^{a}	42.45^{c}	0.54	<0.01	<0.01	0.21	<0.01
PUFA[15]	19.90	20.67	21.70	21.92	21.02	0.64	0.19	0.04	0.23	0.34
Omega-3 FA	0.73^{a}	0.80^{b}	0.83^{b}	0.82^{b}	0.78^{ab}	0.03	0.06	0.02	0.71	0.03
Omega-6 FA	19.17	19.87	20.86	21.10	20.24	0.61	0.20	0.04	0.22	0.37
PUFA/SFA[16]	0.61	0.62	0.64	0.61	0.63	0.03	0.95	0.77	0.79	0.96
Omega-3/omega-6	0.04^{a}	0.04^{c}	0.04^{bc}	0.04^{ab}	0.04^{a}	0.00	0.01	0.06	0.06	0.01
MUFA/SFA[17]	1.35^{c}	1.24^{b}	1.20^{b}	1.06^{a}	1.28^{bc}	0.04	<0.01	0.01	0.19	0.20
UFA/SFA[18]	1.96^{b}	1.86^{b}	1.83^{b}	1.67^{a}	1.91^{b}	0.07	0.05	0.08	0.41	0.43
IA[19]	0.40	0.41	0.40	0.42	0.38	0.01	0.27	0.85	0.57	0.54

[1] Thermoneutral *ad libitium*
[2] Thermoneutral pair-fed
[3] Heat stress *ad libitium*
[4] Heat stress sterculic oil
[5] Heat stress chromium
[6] Treatment
[7] All heat stress treatments
[8] Total unidentified peaks
[9] Iodine value
[10] Δ^9 desaturase index (C16:0)
[11] Δ^9 desaturase index (C18:0)
[12] Total Δ^9 desaturase index
[13] Total saturated fatty acids
[14] Total monounsaturated fatty acids
[15] Total polyunsaturated fatty acids
[16] Polyunsaturated to saturated fatty acid ratio
[17] Monounsaturated to saturated fatty acid ratio
[18] Unsaturated to saturated fatty acid ratio
[19] Index of Atherogenicity

Table 7B. Average adipoycyte area (μm^2) for each treatment

	Treatments						*P*			
Adipose Depot	TNAL[1]	TNPF[2]	HSAL[3]	HSSO[4]	HSCr[5]	SEM	Trt[6]	TNAL vs. HS[7]	TNPF vs. HS	TNAL vs. TNPF
Abdominal	3299^{bc}	2431^{a}	3796^{c}	2970^{ab}	2888^{ab}	266	0.01	0.88	0.02	<0.01
Inner SubQ	2318	2052	2265	2025	2226	180	0.73	0.51	0.51	0.32
Outer SubQ	2289	2143	2049	2136	1978	93	0.18	0.03	0.45	0.29

[1]Thermalneutral *ad libitum*
[2]Thermalneutral pair-fed
[3]Heat stress *ad libitum*
[4]Heat stress sterculic oil
[5]Heat stress chromium
[6]Treatment
[7]All heat stress treatments

Table 8B. Effects of treatments on moisture content (%) in three adipose depots

	Treatments						*P*			
Adipose Depot	TNAL[1]	TNPF[2]	HSAL[3]	HSSO[4]	HSCr[5]	SEM	Trt[6]	TNAL vs. HS[7]	TNPF vs. HS	TNAL vs. TNPF
Abdominal	5.88	7.28	7.65	8.78	6.62	0.80	0.14	0.09	0.70	0.01
Inner SubQ	9.80	10.98	10.80	11.92	11.44	0.67	0.25	0.06	0.62	0.07
Outer SubQ	12.14[a]	14.91[d]	13.03[ab]	13.12[abc]	13.92[bcd]	0.69	0.08	0.16	0.04	0.01

[1]Thermalneutral *ad libitum*
[2]Thermalneutral pair-fed
[3]Heat stress *ad libitum*
[4]Heat stress sterculic oil
[5]Heat stress chromium
[6]Treatment
[7]All heat stress treatments

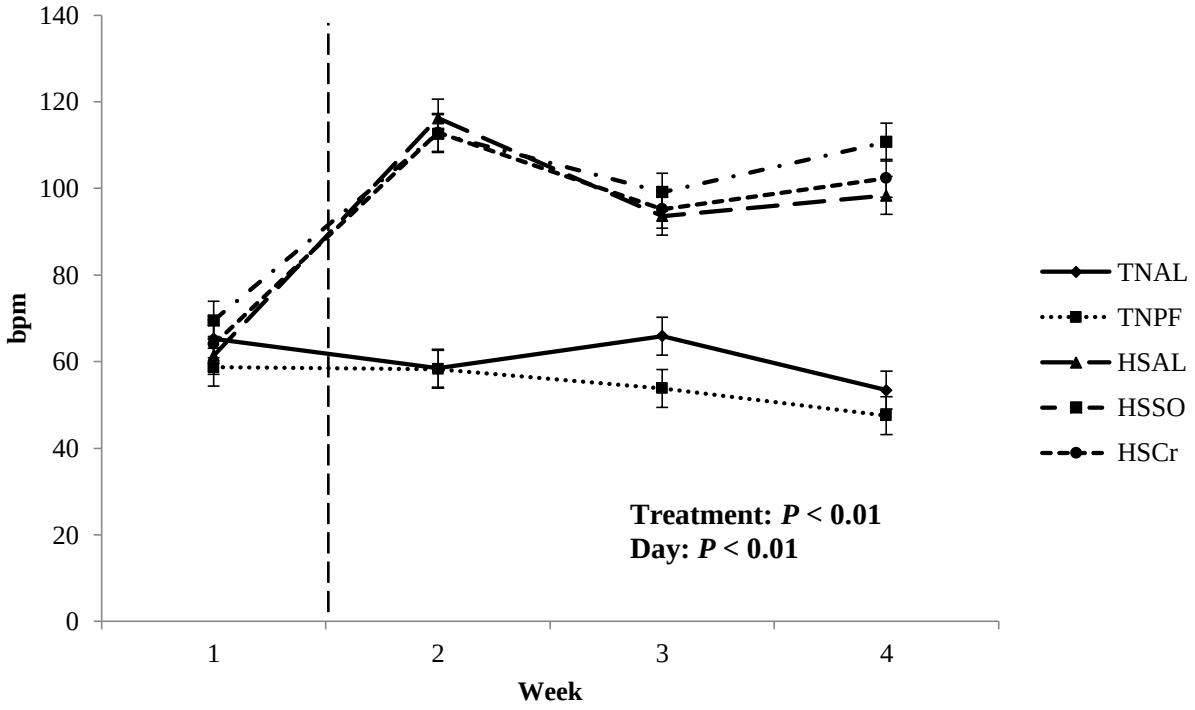

Figure 1. *Effect TNAL, TNPF, HSAL, HSSO, or HSCr on PM respiration rate during the study*

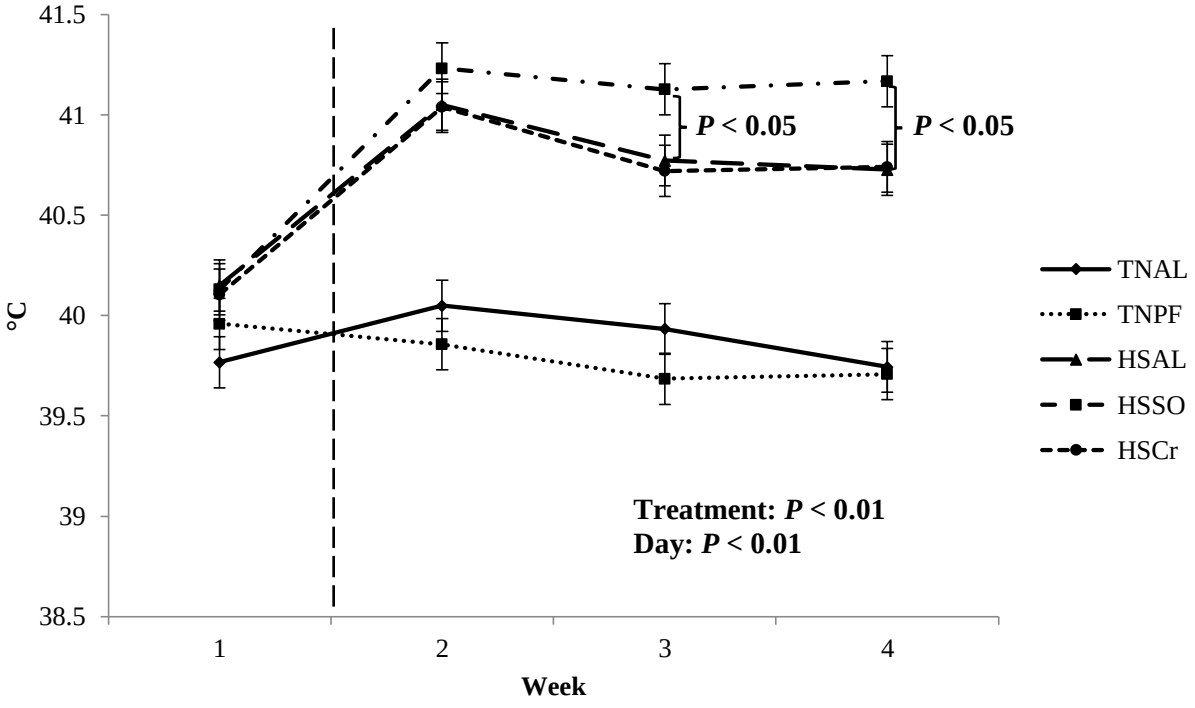

Figure 2. *Effect of TNAL, TNPF, HSAL, HSSO, or HSCr on PM rectal temperature during the study*

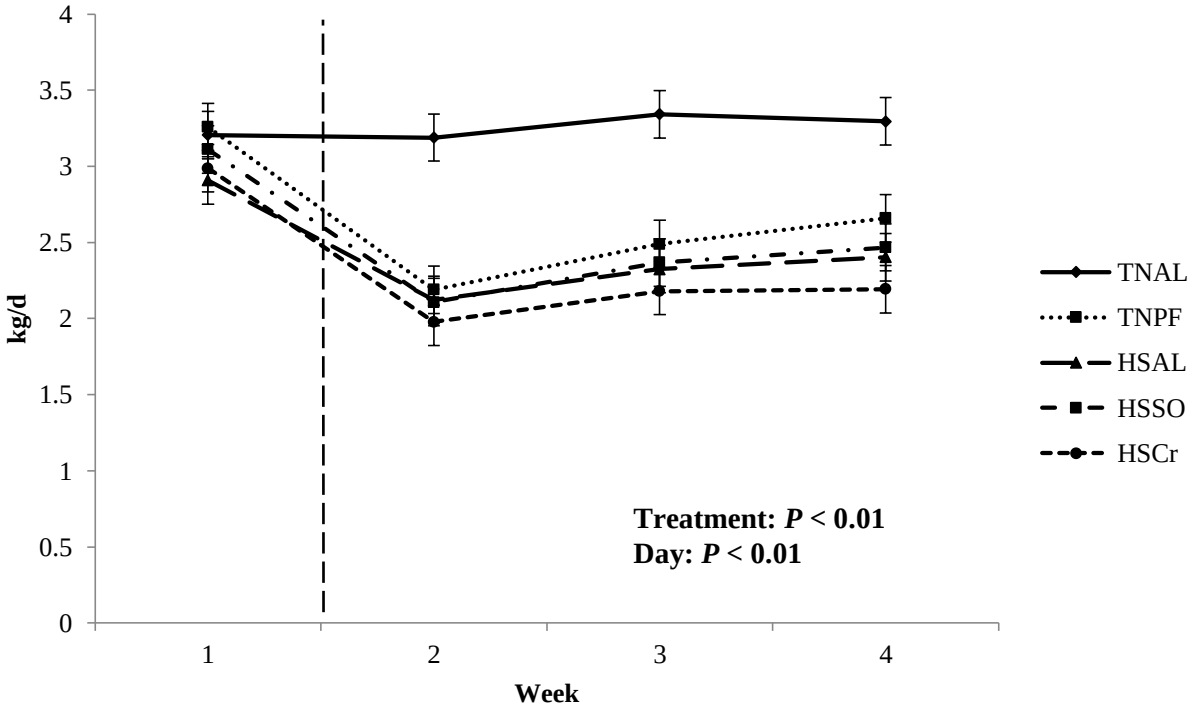

Figure 3. *Effect TNAL, TNPF, HSAL, HSSO, or HSCr on feed intake during the study*

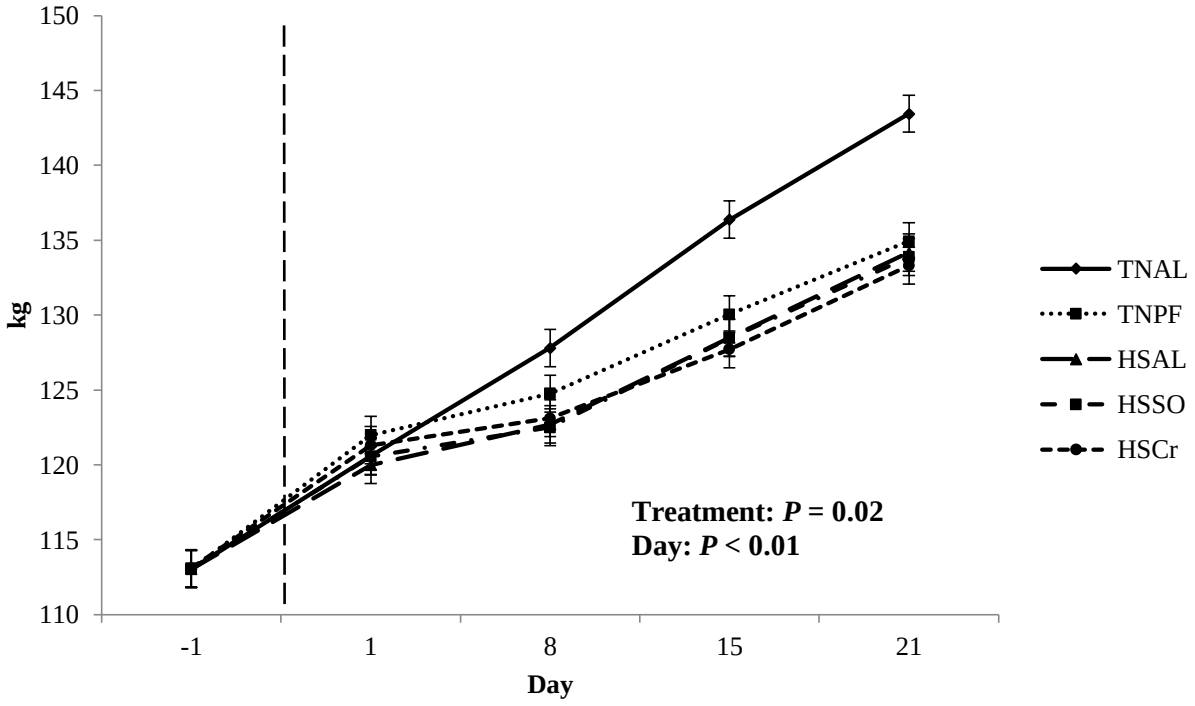

Figure 4. *Effect of TNAL, TNPF, HSAL, HSSO, or HSCr on body weight during the study*

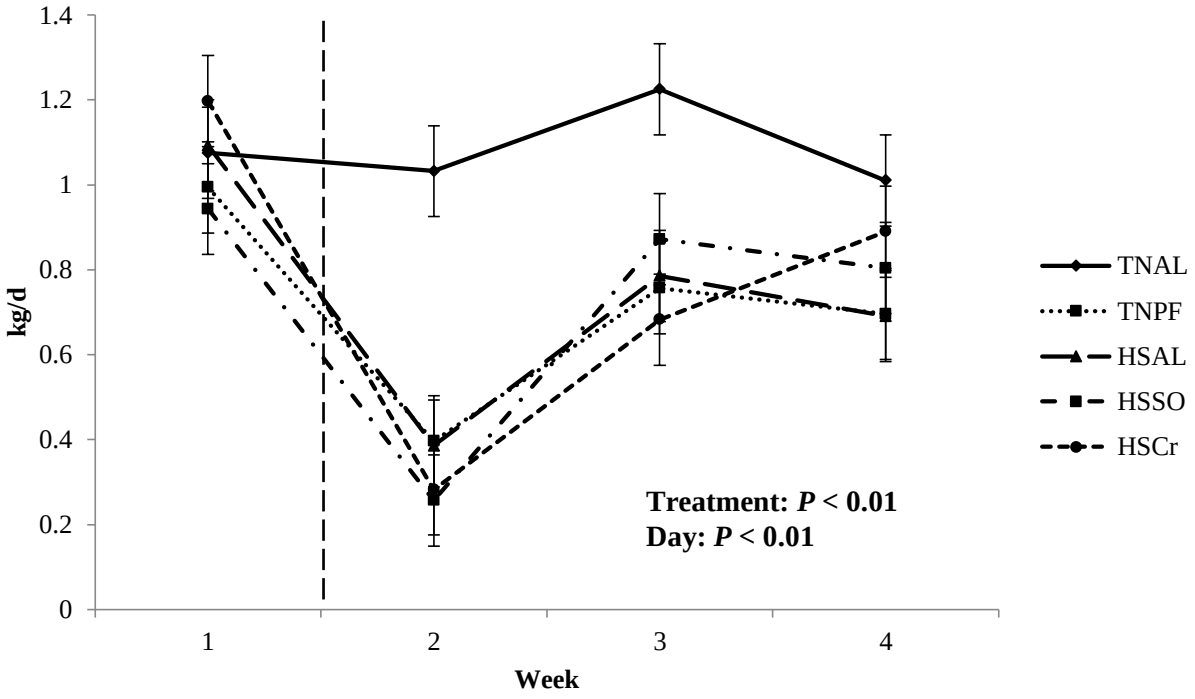

Figure 5. *Effect TNAL, TNPF, HSAL, HSSO, or HSCr on average daily gain during the study*

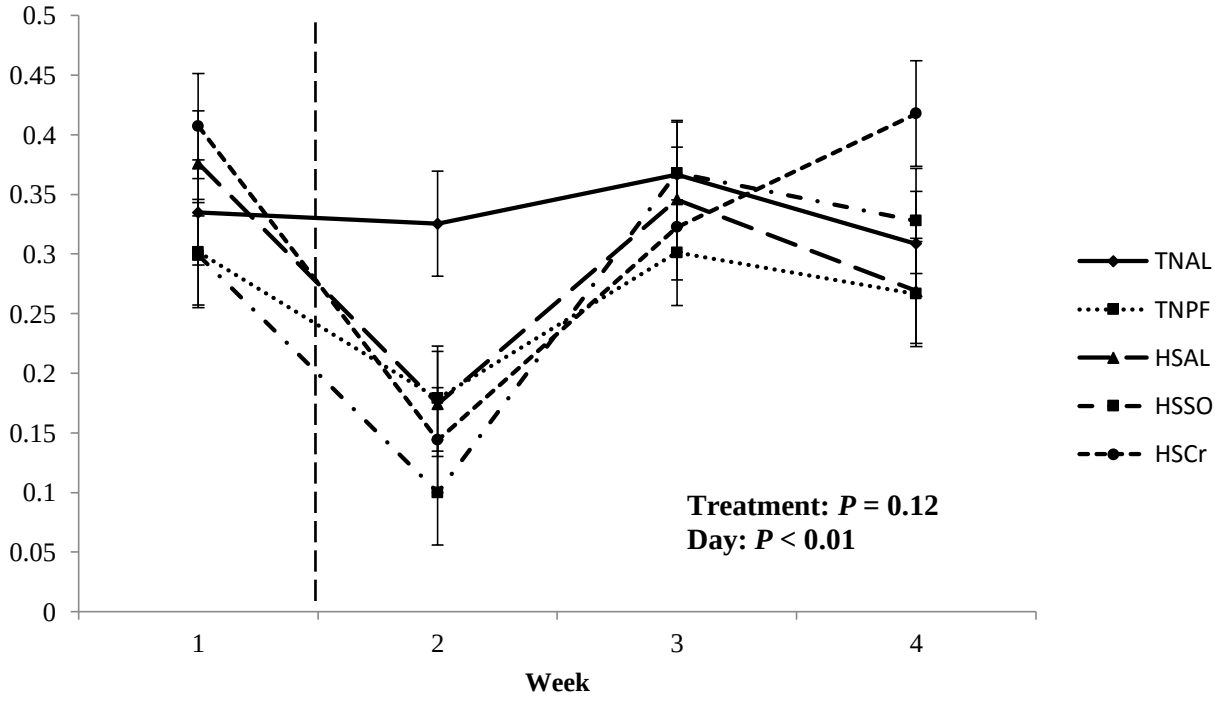

Figure 6. *Effect of TNAL, TNPF, HSAL, HSSO, or HSCr on feed efficiency during the study*

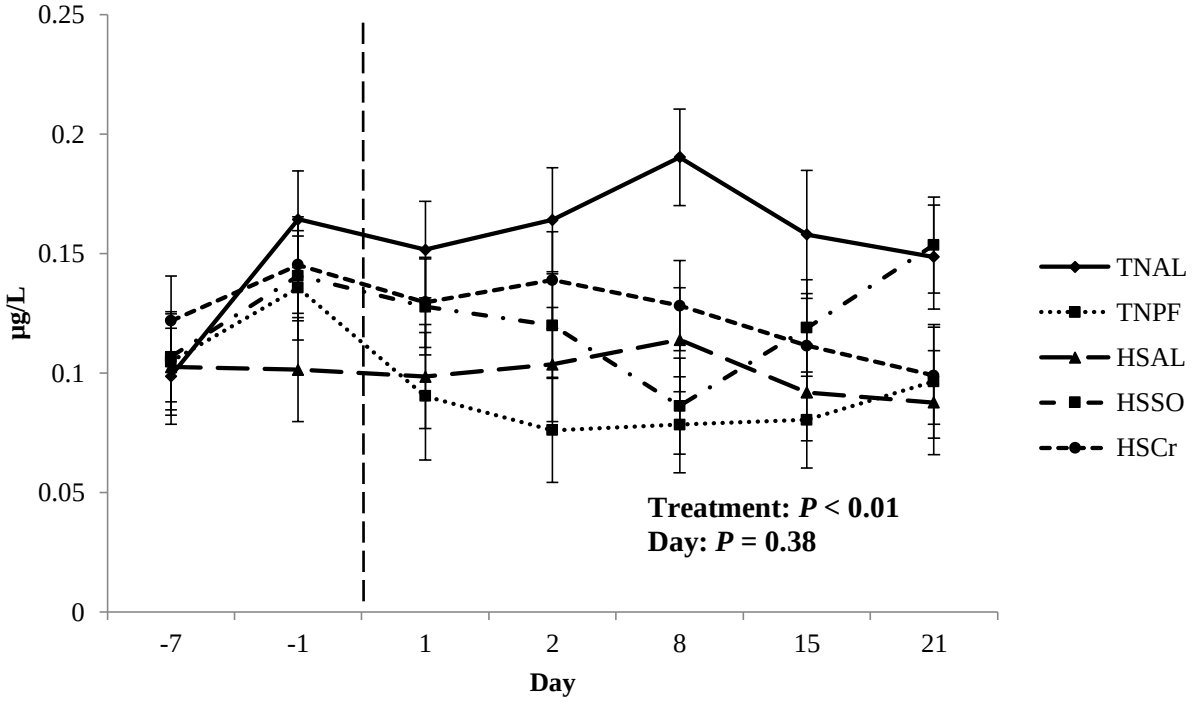

Figure 7. *Effect TNAL, TNPF, HSAL, HSSO, or HSCr on blood insulin levels during the study*

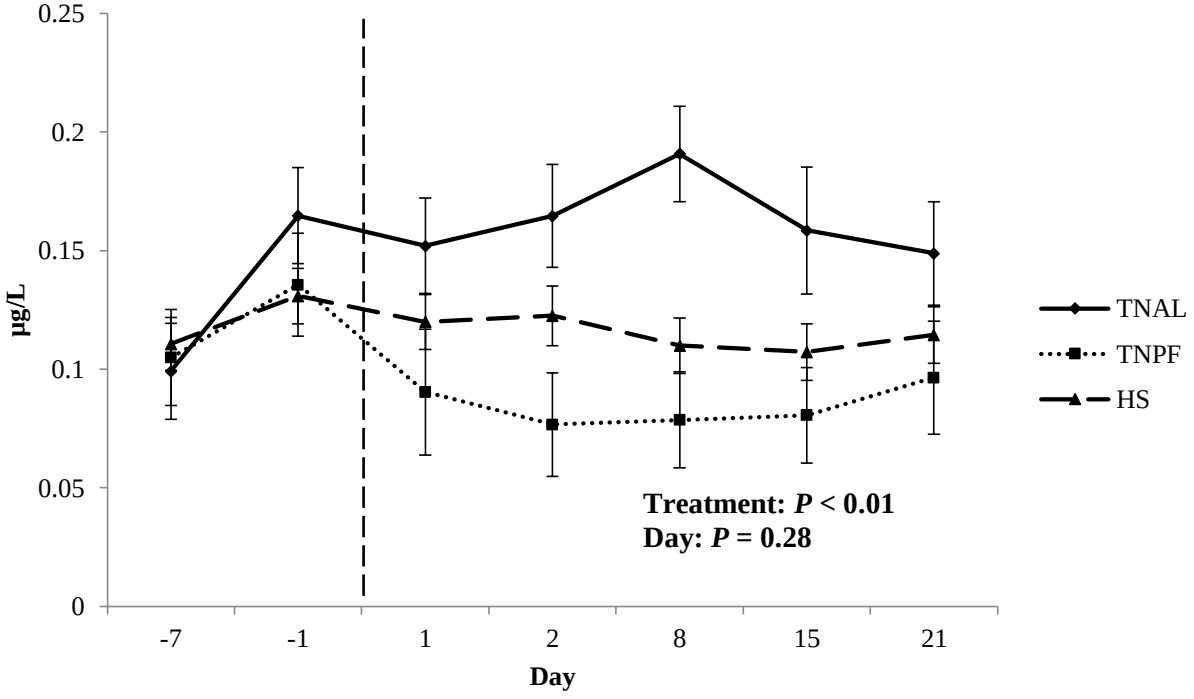

Figure 8. *Effect TNAL, TNPF, HSAL, HSSO, or HSCr on blood insulin levels during the stud*

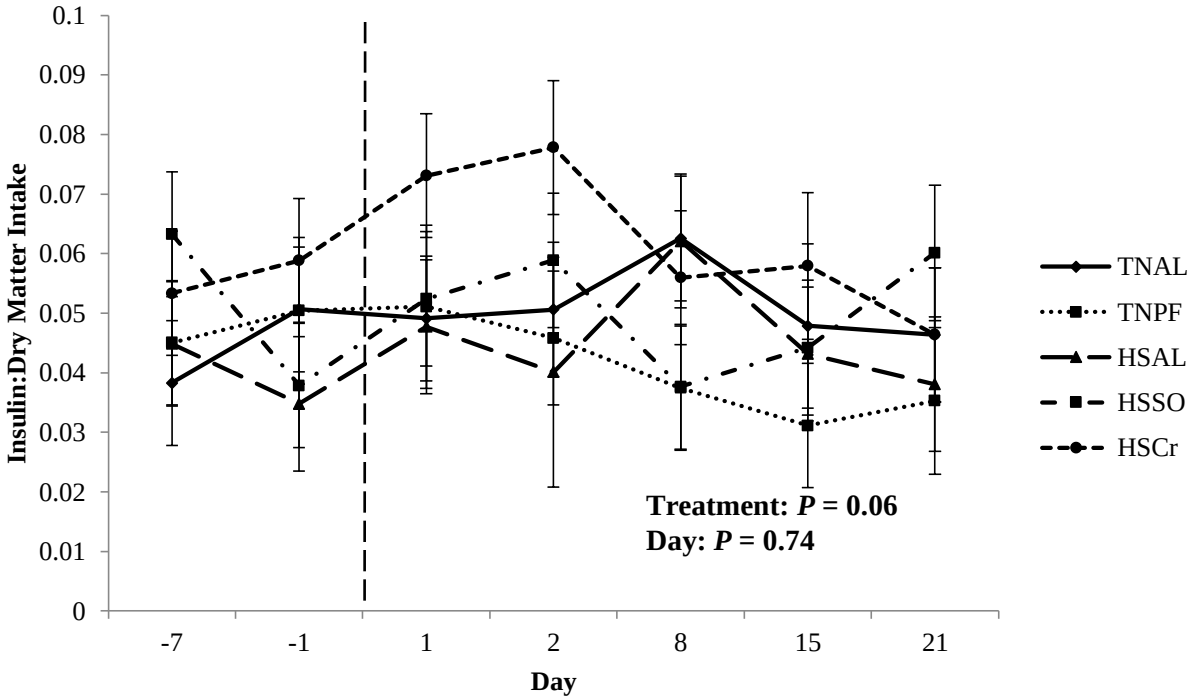

Figure 9. *Effect TNAL, TNPF, HSAL HSSO, or HSCr on insulin:dry matter intake during the study*

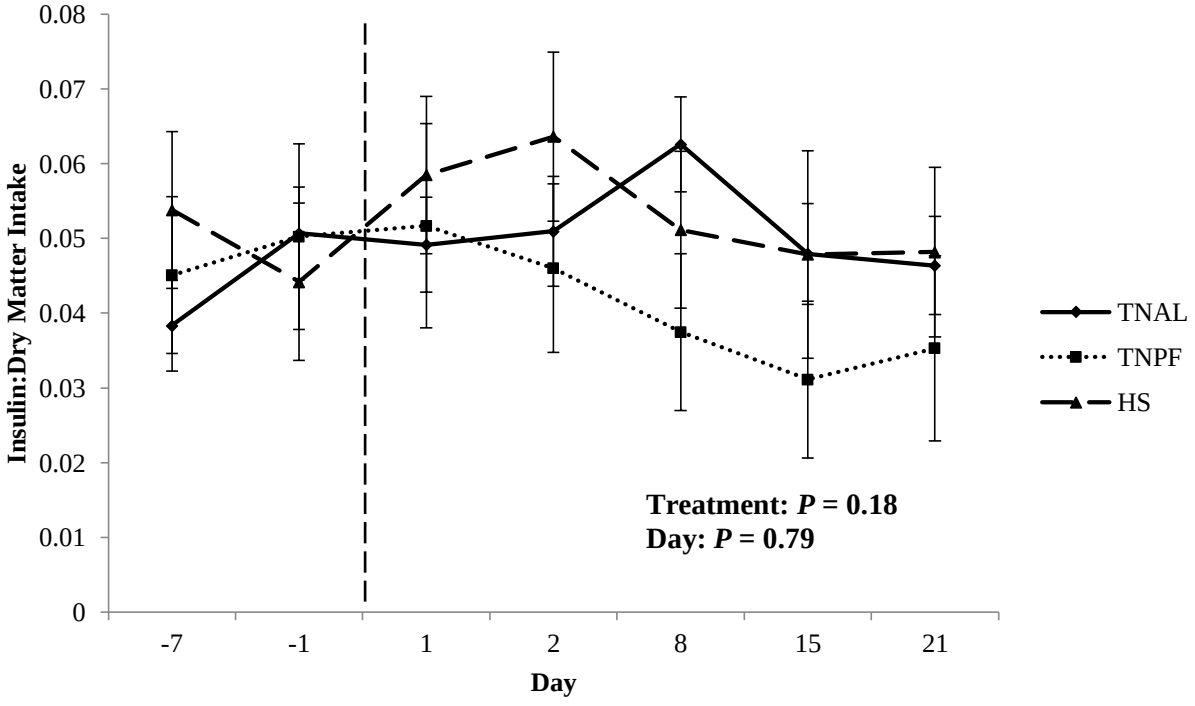

Figure 10. *Effect TNAL, TNPF, HSAL, HSSO, or HSCr on insulin:dry matter intake during the study*

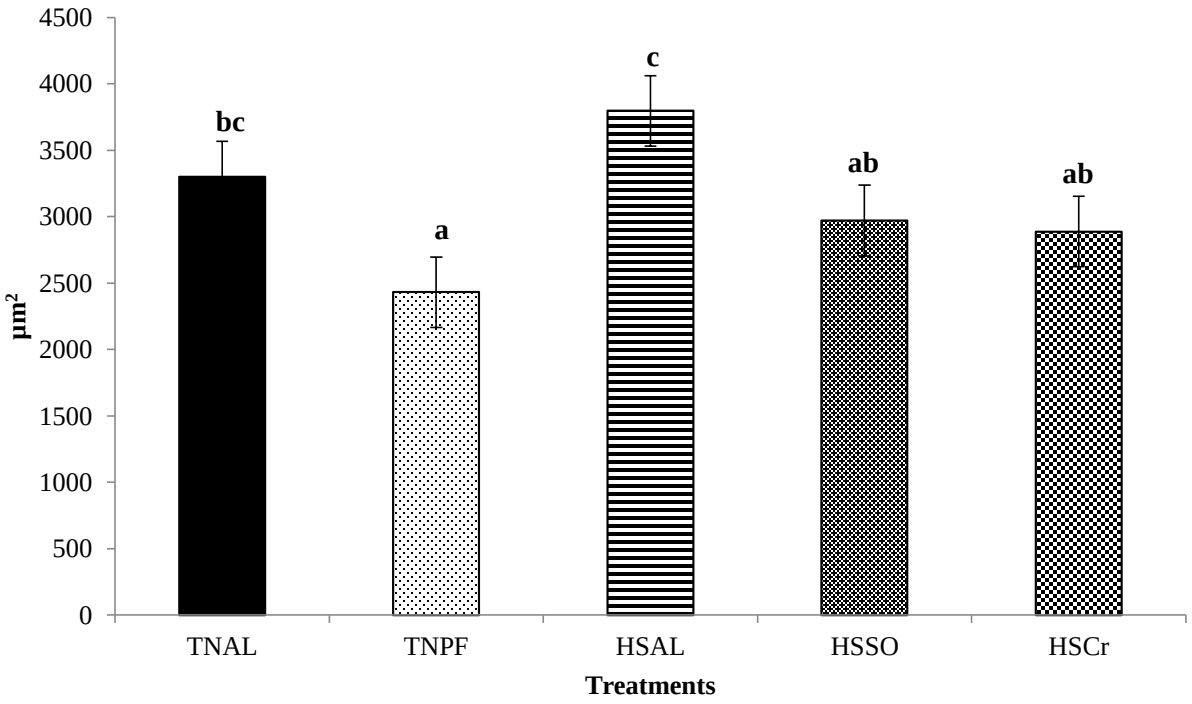

Figure 11. *Effect TNAL, TNPF, HSAL, HSSO, or HSCr on abdominal adipose adipocyte area*

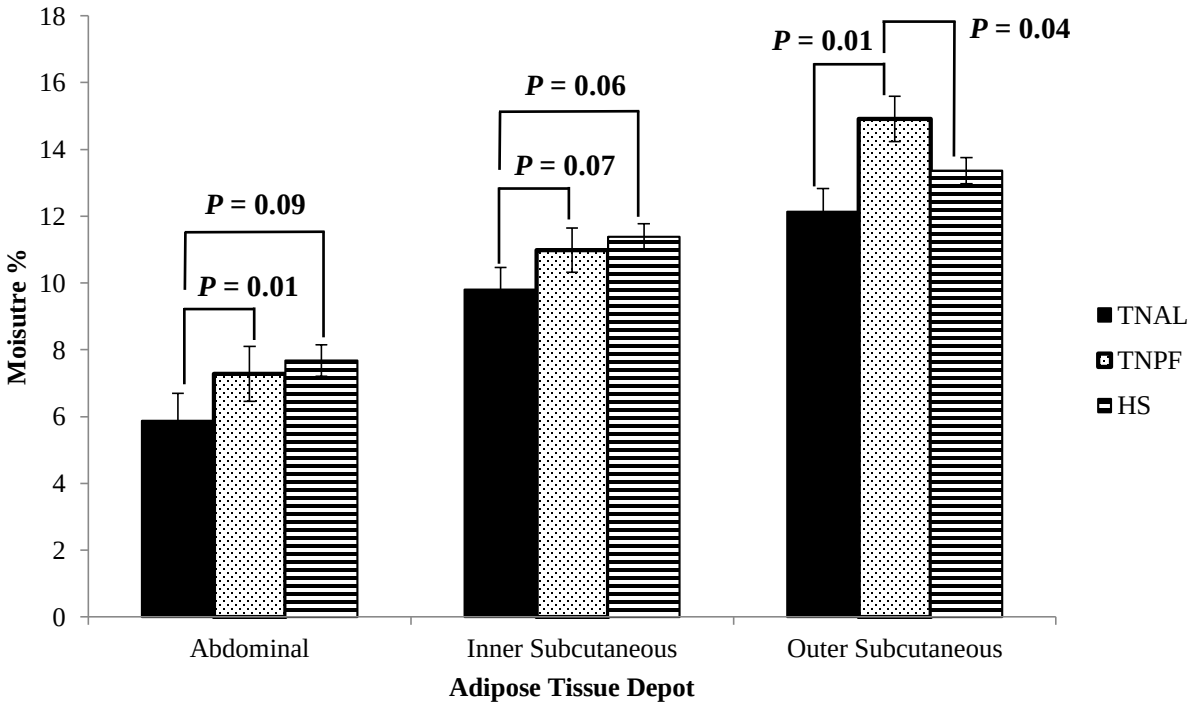

Figure 12. *Effect TNAL, TNPF, HSAL, HSSO, or HSCr on adipose tissue moisture conten*

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