

## Physiology and Endocrinology: Physiology of Heat Stress

**TH161 Thermal and nutritional regulation of hepatic gluconeogenic genes in growing beef cattle.** M. D. O'Brien\*, L. C. Cole, J. B. Wheelock, S. R. Sanders, G. C. Duff, L. H. Baumgard, and R. P. Rhoads, *University of Arizona, Tucson.*

The effect of heat stress (HS) on hepatic expression of key gluconeogenic genes in growing beef cattle was examined and a pair-fed (PF) design was used to differentiate HS effects from those related to reductions in feed intake (FI) typically associated with HS. Holstein bull calves (134 ± 4 kg) were housed in climate-controlled chambers, fed an 86% concentrate, 14% protein diet and subjected to two experimental periods: 1) thermal-neutral (TN; 18-20°C) with ad libitum intake for 9 d and 2) HS (cyclical daily temperatures ranging from 29.4°C to 40.0°C) with ad libitum intake or PF (thermal-neutral conditions) for 9 d. Throughout the study, individual FI was measured on a daily basis whereas rectal temperature (RT) and respiration rate (RR) were measured at four h intervals. Liver biopsies were obtained on d 9 of each period. Hepatic total RNA was isolated, cDNA synthesized and real-time PCR analysis performed. During HS, RT and RR increased (39.0 to 40.6°C and 42 to 126 breaths/min;  $P < 0.01$ ) and DMI decreased by 12%. By design, PF bulls received a similar plane of nutrition. Pyruvate carboxylase (PC) gene expression increased ( $P < 0.05$ ) following HS exposure but remained unchanged during PF. Neither HS nor PF affected expression of the cytosolic phosphoenolpyruvate carboxykinase 1 (PCK) gene. Abundance of peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) mRNA, a transcriptional coactivator that acts at the promoter level of gluconeogenic genes such as PC and PCK, was measured. Despite altered PC gene expression, PGC-1 $\alpha$  mRNA abundance was unaffected by HS. Similarly, reduced FI during PF did not alter PGC-1 $\alpha$  gene expression. In summary, modulation of PC gene expression by HS appears to occur via mechanisms which are independent of reduced nutrient intake.

**Key Words:** Heat Stress, Cattle, Hepatic Gluconeogenesis

**TH162 Seasonal differences in gene expression in oocytes from Holstein cows in a subtropical environment as revealed by gene array analysis.** P. J. Hansen\*<sup>1</sup>, M. Salem<sup>2</sup>, A. M. Brad<sup>1</sup>, J. Yao<sup>2</sup>, and G. W. Smith<sup>3</sup>, <sup>1</sup>University of Florida, Gainesville, <sup>2</sup>West Virginia University, Morgantown, <sup>3</sup>Michigan State University, East Lansing.

In dairy cows, oocyte competence for development after fertilization or electrical activation is reduced during and after heat stress. There is evidence that follicular function can be reduced by heat stress as early as ~30 d before ovulation. The molecular basis for the effect of heat stress on oocyte function is not well understood. Here, gene array analysis was used to identify differences in RNA transcript profiles between oocytes collected in summer and winter. Cumulus-oocyte complexes (COCs) were harvested from ovaries of Holstein cows collected at an abattoir in Florida. Only those with at least 4-5 layers of cumulus cells and evenly granulated oocyte cytoplasm were collected. Oocytes were denuded of cumulus cells and stored at -80°C in pools of ~20. A total of six pools were collected in summer (July-Aug) and six pools in winter (Jan-Feb). RNA was extracted from each pool, subjected to linear amplification and amplified RNA from each pool labeled and hybridized using a two-color, dye swap design to a bovine array consisting of 8,000 unique, 70-mer oligos representing predicted bovine mRNAs. Initial data analysis

revealed 105 and 44 transcripts with greater abundance in summer and winter, respectively ( $P < 0.01$ ) and mapping to distinct gene ontology categories. Further analysis incorporating a false discovery rate of 6 % revealed 7 transcripts displaying greater abundance during summer. Three encode for genes of unknown function. Known genes encoding for transcripts of increased abundance during summer included glutathione S-transferase subunit isoform I, which participates in repair of oxidative stress damage, cyclin D2, which can activate CDK kinases involved in cell proliferation and block inhibition of cell cycle caused by retinoblastoma protein, PRA1 family protein 2, which is involved in protein trafficking, and 17-beta-hydroxysteroid dehydrogenase type 1, which is involved in steroid metabolism. Results are consistent with summer conditions causing an increase in transcripts for proteins that act to stabilize oocyte function during thermal stress. Support: BARD US3986-07

**Key Words:** Heat Stress, Oocyte, Gene Array

**TH163 The effect of heat stress and exogenous bovine somatotropin on expression of genes associated with hepatic gluconeogenesis in lactating dairy cows.** J. B. Wheelock\*, A. J. LaNoce, M. D. O'Brien, S. R. Sanders, R. J. Collier, L. H. Baumgard, and R. P. Rhoads, *University of Arizona, Tucson.*

Recent evidence indicates heat stress (HS) alters whole-body glucose metabolism independently of HS-induced reductions in feed intake. The majority of bovine whole-body glucose demands are met by hepatic gluconeogenesis however it is unknown whether HS affects liver glucose production. Therefore, study objectives were to examine hepatic expression of key gluconeogenic genes in lactating dairy cows during HS or in thermal neutral pair-fed animals (PF) and administered bovine somatotropin (rbST) in each condition. Multiparous (99 DIM) Holstein cows [n = 10 (HS), n = 12 (PF)] were subjected to three 7 d experimental periods: 1) thermal neutral, ad libitum intake, 2) HS or PF, and 3) HS or PF with rbST (POSILAC<sup>®</sup>) administered on d 1 of period 3. HS temperatures were cyclical, ranging from 29.7 to 39.2°C. Milk yield, DMI, respiration rate (RR) and rectal temperature (RT) were measured daily. Liver biopsies were obtained on d 7 of each period for total RNA isolation, cDNA synthesis and real-time PCR analysis. Heat stress increased RR and RT and reduced DMI by 30%. By design, PF cows received a similar plane of nutrition. During HS and PF, milk yield decreased by 27.5 and 15.3%, respectively and increased following rbST administration (~13%). During PF, pyruvate carboxylase (PC) and cytosolic phosphoenolpyruvate carboxykinase (PCK) gene expression increased (60%,  $P < 0.05$ ) or tended to increase (40%,  $P < 0.10$ ), respectively. In contrast, PC gene expression increased by 45% ( $P < 0.05$ ) but PCK mRNA abundance was unaltered by HS. Similarly, peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) mRNA abundance tended to increase ( $P < 0.10$ ) during PF but was unaffected by HS. Administration of rbST did not alter gluconeogenic mRNA abundance despite causing a 2- and 3-fold increase ( $P < 0.05$ ) in insulin-like growth factor-I (IGF-I) gene expression during HS and PF, respectively. In summary, HS leads to a change in hepatic gluconeogenic gene expression that appears independent of reduced feed intake.

**Key Words:** Heat Stress, Gluconeogenesis, Cattle

**TH164 Heat shock modulates adipokines secretion in 3T3-L1 adipocytes.** U. Bernabucci\*, L. Basirico, P. Morera, N. Lacetera, B. Ronchi, and A. Nardone, *Dipartimento di Produzioni Animali, Viterbo, Italy.*

Several studies have demonstrated that high temperatures are responsible for modifications of lipid/carbohydrate metabolism in farm animals, but mechanisms are not yet clarified. Adipose tissue acts through secretory factors called adipokines (adiponectin and leptin) that are expressed in differentiated adipocytes and are involved in modulation of both lipid and glucose metabolism. To date there is no information concerning the possible relationship between heat stress and adipokines expression. Therefore, the objective of the present study was to investigate the effect of heat shock on adiponectin and leptin secretion in 3T3-L1 adipocytes. Adipocytes were incubated at different temperatures: 37 (control), 39 and 41°C. For each temperature, samples were collected after 0, 2, 4, 8, 16 and 24h of exposure. Adiponectin and leptin secretion were measured in cell culture media using commercial ELISA kits. As molecular markers of cell injury Hsp70.2 gene expression was determined by real time-PCR. Cell viability was determined by XTT assay. Heat shock affected adipokines secretion. Compared with 37°C, secretion of adiponectin increased ( $P \leq 0.01$ ) at 39°C, while at 41°C, the protein was not detectable. Unlike adiponectin, the concentration of leptin increased ( $P \leq 0.01$ ) at 41°C. The Hsp-70.2 gene expression was increased ( $P \leq 0.05$ ) already at 39°C and the higher level ( $P \leq 0.01$ ) was reached at 41°C compared with 37°C. Exposure to different temperatures did not affect cell viability. Results of the present study show that mild heat stress (39°C) caused an increase in adiponectin secretion, while severe heat stress (41°C) caused upregulation of leptin and downregulation of adiponectin. The study provides the first evidence of a direct modulation of heat shock on adiponectin and leptin secretion in 3T3-L1 adipocytes. Changes in leptin and adiponectin induced by severe heat stress might be involved in the alteration of lipid/carbohydrate metabolism occurring in animals exposed to a hot environment.

**Key Words:** Heat Shock, 3T3-L1, Adipokines

**TH165 Effects of elevated ambient temperature on length of gestation and ruminal temperature at parturition of beef cows.** E. C. Wright\*, M. J. Prado-Cooper, C. L. Bailey, and R. P. Wettemann, *Oklahoma Agricultural Experiment Station, Stillwater, OK.*

Angus x Hereford cows ( $n = 27$ ) were randomly assigned to four groups and AI to calve in mid August (MAug), late August (LAug), mid September (Sept) or mid October (Oct) to evaluate the effects of elevated ambient temperature on length of gestation and ruminal temperature at parturition. Temperature boluses (SmartStock, LLC) were placed in the rumen at 255 d of gestation. Boluses were programmed to transmit temperature every hr. Cows grazed native pasture in Oklahoma and had a body condition score of  $6.5 \pm 0.4$  at calving. Length of gestation in five previous years were shorter ( $277.7 \pm 1.4$  d;  $P = 0.01$ ) for cows that calved in August compared with cows that calved in October ( $281.8 \pm 2.3$  d). Maximum ambient temperatures during the week before the expected calving date were greater for MAug ( $34.1 \pm 2.3^\circ\text{C}$ ) and LAug ( $34.0 \pm 2.7^\circ\text{C}$ ,  $P < 0.001$ ) compared with Sept ( $29.7 \pm 3.5^\circ\text{C}$ ) and Oct ( $28.5 \pm 3.2^\circ\text{C}$ ). Length of gestation was shorter for cows in MAug ( $274.7 \pm 5.5$  d,  $P = 0.05$ ) compared with Oct ( $278.8 \pm 3.1$  d), but did not differ from LAug ( $277.0 \pm 2.5$  d,  $P = 0.29$ ) and Sept ( $276.2 \pm 3.1$  d,  $P = 0.50$ ). Ruminal temperature during the week before calving was not influenced by month of calving ( $P = 0.84$ ) and averaged  $38.8 \pm 0.27^\circ\text{C}$  for all months. Concentrations of cortisol in plasma 1 and 2 d before parturition were less for LAug ( $6.3 \pm 5.7$  ng/mL) compared with MAug ( $10.8 \pm 5.9$  ng/mL,  $P = 0.03$ ) and Oct ( $12.5 \pm 4.7$  ng/mL,  $P = 0.003$ ) cows. Exposure of beef cows to elevated ambient temperature results in shorter gestations.

**Key Words:** Gestation Length, Parturition, Beef Cow