Cattle temperament influences metabolism: Metabolic response to glucose tolerance and insulin sensitivity tests in beef steers^{*,†}

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ABSTRACT

Cattle temperament, defined as the reactivity of cattle to humans or novel environments, can greatly influence several physiological systems in the body, including immunity, stress, and most recently discovered, metabolism. Greater circulating concentrations of non-esterified fatty acids (NEFAs) found in temperamental cattle suggests that temperamental cattle are metabolically different than calm cattle. Further, elevated NEFA concentrations have been reported to influence insulin sensitivity. Therefore, the objective of this study was to determine whether cattle temperament would influence the metabolic response to a glucose tolerance test (GTT) and insulin sensitivity test (IST). Angus-cross steers (16 Calm and 15 Temperamental; 216 ± 6 kg BW) were selected based on Temperament Score measured at weaning. On day 1, steers were moved into indoor stanchions to allow measurement of individual *ad libitum* feed intake. On day 6, steers were fitted with indwelling rectal temperature probes and jugular catheters. At 0900 h on day 7, steers received the GTT (0.5 mL/kg BW of a 50% dextrose solution) and at 1400 h on day 7, steers received the IST (2.5 IU bovine insulin/kg BW). Blood samples were collected and serum isolated at -60, -45, -30, -15, 0, 10, 20, 30, 45, 60, 90, 120, and 150 min relative to each challenge. Serum was stored at -80°C until analyzed for cortisol, glucose, NEFA, and blood urea nitrogen (BUN) concentrations. All variables changed over time ($P < 0.01$). For the duration of the study, Temperamental steers maintained greater ($P < 0.01$) serum NEFA and less ($P \leq 0.01$) serum BUN and insulin sensitivity (calculated using RQUICKI) compared to Calm steers. During the GTT, Temperamental steers had greater ($P < 0.01$) serum glucose, yet decreased ($P = 0.03$) serum insulin, and ($P < 0.01$) serum insulin: serum glucose compared to Calm cattle. During the IST, Temperamental steers had greater ($P < 0.01$) serum insulin and a greater ($P < 0.01$) serum insulin: serum glucose as compared to Calm steers. These data demonstrate that differences exist in the manner in which Temperamental steers respond to glucose and insulin,

36 potentially a result of elevated serum NEFA concentrations, which may result in changes in
37 utilization and redistribution of energy in Temperamental versus Calm cattle.

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39 *Keywords:* Cattle, Glucose, Insulin, Insulin sensitivity, Non-esterified fatty acids, Temperament

1. Introduction

Cattle temperament, defined as the reactivity of cattle to humans or novel environments [1], can influence several physiological systems in the body, including immunity, stress, and most recently discovered, metabolism. For example, temperamental cattle display limited or no sickness behavior in response to an endotoxin challenge, and temperamental cattle have greater basal body temperature than calm cattle [2-4]. Additionally, temperamental cattle have greater basal cortisol and catecholamine (i.e., epinephrine and norepinephrine) concentrations, yet exhibit a blunted cortisol response to corticotropin releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), and endotoxin challenges [5-7] compared to calm cattle. There have also been reports that temperamental cattle have an altered pro-inflammatory cytokine response to endotoxin challenge [8] and have decreased antibody titer responses to vaccination [9] compared to calm cattle; however, this has yet to be corroborated. Lastly, several performance parameters (ADG, carcass bruise score, and marbling) are altered in a negative manner in temperamental compared to calm cattle [1, 10-13], which contributes to the decision of producers to select against temperamental cattle.

More recently, the authors reported differences in metabolism between temperamental and calm cattle [5]. In this study, temperamental cattle maintained greater circulating concentrations of NEFAs and decreased serum blood urea nitrogen (BUN), but temperamental cattle appear to be less sensitive to insulin [5]. Research in models of human diabetes and in the early lactation dairy cow demonstrate that elevated NEFA concentrations can influence the manner in which the body responds to glucose and insulin [14, 15]. Specifically, elevated NEFAs may result in decreased insulin sensitivity and reduced glucose uptake, which can ultimately influence downstream energy requirements and repartitioning of energy resources [14,

16, 17]. Based on these data, we hypothesized that the greater serum NEFA concentrations observed in temperamental cattle may influence other metabolic parameters including those involved with glucose and insulin. Therefore, this study was designed to further explore the differential metabolism exhibited by temperamental cattle, and these results are the first to demonstrate altered glucose and insulin dynamics in Temperamental steers in response to glucose tolerance and insulin sensitivity tests.

2. Materials and methods

All experimental procedures were in compliance with the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* and approved by the Institutional Animal Care and Use Committee of the Livestock Issues Research Unit.

2.1. Experimental Design

In March 2014, Angus-cross steers (216 ± 6 kg BW), 16 Calm and 15 Temperamental, were selected from a herd of 250 cattle at the Mississippi Agriculture and Forestry Experiment Station - Brown Loam in Raymond, MS. Steers were selected based on Temperament Score (TS) measured at weaning. Steers were weaned in November 2013. Temperament Score [18] was calculated as an average of exit velocity and pen score (see description below) [5]. Based on TS, 16 steers with the lowest TS and classified as Calm (TS: 2.00 ± 0.15 ; EV: 1.92 ± 0.16 m/s; PS: 2.19 ± 0.19), and 15 steers with the greatest TS and classified as Temperamental (TS: 3.94 ± 0.09 ; EV: 4.21 ± 0.21 m/s; PS: 3.67 ± 0.16) were selected. On day 0, steers were transported from Raymond, MS to Lubbock, TX (approximately 1184 km, 12 h). Steers were group housed in outdoor pens (7.6 x 18.3 m) overnight with access to feed and water. The steers were supplied a feed in Lubbock that was similar to the feed provided in Mississippi. The following day steers

were moved into individual stanchions (2.28 m in length, 0.76 m in width, 1.67 m in height) to allow for measurement of individual *ad libitum* feed intake through day 4. Steers were fed a mixture of 60% certified timothy grass pellets (8% CP, 1.5% crude fat, and 35% crude fiber) and 40% concentrate (12% CP, 3% crude fat, 18% crude fiber) and had free access to water throughout the study. On the afternoon of day 5, steers were moved from the individual stanchions to the outdoor pens with access to the same diet and water. On day 6, steers were weighed, fitted with indwelling jugular catheters and rectal temperature recording devices [19] that measured rectal temperature continuously throughout the study at 5-min intervals. The steers were then returned to the individual stanchions for the remainder of the study. Upon returning to individual stanchions, steers received the daily allotment of feed. On day 7 prior to the glucose tolerance test (0600 h), orts were removed from feed bunks and no feed was offered to the steers until the completion of the insulin sensitivity test.

On day 7 at 0900 h, steers received a glucose tolerance test i.v. (0.5 mL/kg BW 50% dextrose solution). Whole blood samples were collected into Sarstedt tubes containing no additive (Sarstedt, Inc., Newton, NC) at -60 (0800 h), -45, -30, -15, 0, 10, 20, 30, 45, 60, 90, 120, and 150 min (1130 h) relative to the glucose tolerance test. The dextrose bolus was administered immediately after collection of the 0 min sample. On day 7 at 1400 h, steers received the insulin sensitivity test i.v. (2.5 IU/kg BW bovine insulin; Sigma Aldrich, St. Louis, MO; Fig. 1). Whole blood samples were collected into Sarstedt tubes containing no additive (Sarstedt, Inc., Newton, NC) at -60 (1300 h), -45, -30, -15, 0, 10, 20, 30, 45, 60, 90, 120, and 150 min (1630 h) relative to the insulin sensitivity test. The insulin bolus was administered immediately after collection of the 0 min sample. Here and throughout, reference to the glucose tolerance test or insulin sensitivity test refers to the administration of the glucose or insulin bolus.

For blood sample collection, a 3-mL waste sample was collected from the catheter extension (approximately 1.5 m in length, approximately 2 mL in volume), followed by collection of the blood sample (9 mL). Next, 10 mL of sterile saline was administered to replace fluid loss, and 2.5 mL of heparinized saline (10 USP/mL) was administered to maintain catheter patency. The entire sample collection procedure was completed within 2 - 5 min. Whole blood samples were allowed to clot at room temperature for 30 min and were then centrifuged at 1500 g for 20 min at 4°C. Isolated serum was stored at -80°C until analyzed for serum concentrations of cortisol, glucose, insulin, NEFA and BUN.

2.2. Temperament measurements

Temperament score was calculated as an average of exit velocity and pen score measured at weaning. Exit velocity is an objective measurement that represents the rate of speed of a calf traversing a distance of 1.83 m after its exit from a working chute [20, 21]. This was determined using two infrared sensors (FarmTek Inc., North Wylie, TX, USA) and was done by calculating velocity [velocity = distance/time; expressed as m/s]. Pen score [22] is a subjective measurement obtained by separating cattle into small groups of three to five and scoring their reactivity to a human observer. In brief, a human observer approached each calf and assigned a score between 1 and 5 based on the calf's reaction to the observer. A score of one was given to calves that were docile, did not react to the observer, and allowed the observer to approach. A calf given a score of 2 was slightly flighty, was aware of the observer and likely stood in a corner away from the observer. A score of 3 was given to calves that moved away from the observer. These calves would run with a raised head alongside the fence, fully aware of the position of the observer. A calf given a score of 4 was considered flighty. They were aware of the observer and may have

run along the fence or into gates or fences. A score of 5 was given to those considered very flighty. Those calves are often called ‘crazy’ and would often run at gates, fences, and humans in an attempt to exit the pen. Calves given a score of 5 were allowed to exit the pen in order to more accurately determine the scores of the remaining calves (i.e., to avoid the temperamental animals agitating the other calves, especially if they are of a calmer temperament). Pen score was assigned by the same observer, skilled in assigning pen scores on this herd for over 15 years; therefore an inter-observer reliability was not established.

2.3. Serum analysis

All serum analyses were performed in duplicate. Serum cortisol concentrations were determined using a commercially available enzyme immunoassay kit according to the manufacturer’s directions (Arbor Assays, Ann Arbor, MI) by comparison of unknowns to standard curves generated with known concentrations of cortisol. The minimum detectable cortisol concentration was 0.0454 ng/mL, and the intra- and inter-assay coefficients of variation were less than 15.1% and 17.0%, respectively.

Glucose concentrations were determined by modification of the enzymatic Autokit Glucose (Wako Diagnostics, Richmond, VA USA) to fit a 96-well format as previously described [5]. Briefly, 300 µL of prepared working solution was added to 2 µL of serum or prepared standards in a 96-well plate. Plates were incubated at 37°C for 5 min and absorption was recorded at 505 nm. The plate reader used for this assay (BioTek Powerwave 340; BioTek Instruments, Winooski, VT, USA) has an incubating and timing feature and therefore ensured that the sample absorbances were read immediately following the 5-min incubation. Concentrations of glucose were determined by comparing unknown samples to a standard curve

of known glucose concentrations. The minimum detectable concentration was 3.8 mg/dL and the intra- and inter-assay coefficients of variation were less than 14.7% and 12.4%, respectively.

Insulin concentrations were determined by a bovine-specific insulin ELISA according to the manufacturer's instructions (Cat # 80-INSBO-E01; Alpco Diagnostics, Salem, NH, USA). The minimum detectable concentration was 0.100 ng/mL and the intra- and inter-assay coefficients of variation were less than 7.4% and 9.4%, respectively.

Concentrations of NEFAs were determined by modification of the enzymatic HR Series NEFA-HR (2) assay (Wako Diagnostics, Richmond, VA USA) to fit a 96-well format as previously described [5]. Briefly, 200 μ L of the prepared Color Reagent A were added to 5 μ L of serum or prepared standards in a 96-well plate. Plates were incubated at 37°C for 5 min and then the absorbance was read using a spectrophotometer at 550 nm. Next, 100 μ L of prepared Color Reagent B was added to all wells on the 96-well plate. Plates were incubated for an additional 5 min and read for a second time using a plate reader at 550 nm. The plate reader used for this assay has an incubating timing feature and therefore ensured that the sample absorbances were read immediately following the 5-min incubation. A final absorbance was obtained by subtracting the first reading, which was multiplied by a factor of 0.67 to account for changes in volume, from the second reading. The final absorbance values were used for all calculations (i.e., standard curve, sample concentrations). Concentrations of NEFAs were determined by comparing unknown samples to a standard curve of known NEFA concentrations. The minimum detectable concentration was 0.0014 mmol/L and the intra- and inter-assay coefficients of variation were less than 9.0% and 6.7%, respectively.

Serum concentrations of BUN were determined by a colorimetric assay according to the manufacturer's directions (K024-H1; Arbor Assays, Ann Arbor, MI) by comparison of

unknowns to standard curves generated with known concentrations of urea nitrogen. The minimum detectable BUN concentration was 0.065 mg/dL and the intra- and inter-coefficients of variation were less than 4.4% and 6.9%, respectively

2.4. Data calculations

Additional calculations were made in order to study glucose and insulin dynamics. Specifically, serum glucose clearance, serum glucose half-life, and serum glucose: serum insulin were calculated as previously described [23]. From 10 to 30 minutes post glucose infusion, insulin production peaked, causing serum glucose concentrations to sharply decrease. Serum glucose and insulin concentrations returned to baseline values by 90 minutes post glucose infusion. For the IST, serum glucose concentrations reached minimum concentrations by 45 min post insulin infusion, but the clearance rate of glucose may vary among animals [23].

Insulin sensitivity was calculated based on the Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) method as previously described [24]. Briefly, RQUICKI is calculated as $1/[\log(Gb) + \log(Ib) + \log(FFAb)]$ where Gb is serum glucose concentration in mg/dL, Ib is serum insulin in $\mu\text{U/mL}$, and FFAb is free fatty acids (i.e, NEFA values) in mmol/L. Based on this index, less insulin sensitivity is demonstrated by lower values.

2.5. Statistical analysis

Data were analyzed by the MIXED procedure of SAS specific for repeated measures (SAS Inst. Inc., Cary, NC USA) within each challenge (GTT, and IST). Temperament, time, and the temperament x time interaction were included as fixed effects with steer within temperament group as the experimental unit. Data were analyzed separately in two different time periods:

Baseline, time period prior to application of challenge (-60 to 0 min); and Challenge, time period following administration of the dextrose or insulin bolus (0 to 150 min). When the temperament x time interaction was significant, means were separated using the PDIFF option in SAS, with $P \leq 0.05$ considered significant and $P \leq 0.10$ considered a tendency. All data are presented as the LSM $\pm$ SEM.

3. Results

3.1. Glucose tolerance test

Basal feed intake, measured from day 1 - 4, did not differ ($P = 0.15$; 5.1 ± 0.2 and 4.6 ± 0.2 kg/day for Calm and Temperamental, respectively) between Calm and Temperamental steers. There were differences in body weight between the temperament groups. Specifically, Temperamental cattle weighed more on average throughout the study (226 ± 4 kg; $P < 0.001$) compared to Calm steers (203 ± 4 kg). Additionally, Temperamental steers lost more weight on average (-7.1 ± 1.1 kg; $P = 0.004$) than Calm steers (-2.4 ± 1.1 kg) throughout the study.

Prior to the challenge, there was no effect of temperament, time or a temperament x time interaction ($P \geq 0.82$) on steer rectal temperature (Table 1). Following administration of the dextrose bolus, there was no temperament x time interaction ($P = 1.00$); however, there was an effect of time ($P = 0.001$). Specifically, rectal temperature values decreased at 85 min ($P = 0.03$; $38.77 \pm 0.05^\circ\text{C}$) and remained below 0 min values through the remainder of the challenge ($P \leq 0.03$). Also, there was a tendency ($P = 0.10$) for Calm cattle to have greater rectal temperature post-challenge compared to Temperamental cattle (38.83 vs. $38.80 \pm 0.01^\circ\text{C}$).

Serum cortisol concentrations prior to the challenge were not affected by temperament or a temperament x time interaction (Table 1; $P \geq 0.29$), but were affected by time ($P < 0.001$). Serum cortisol values increased from -60 to -45 min (10.54 vs. 15.39 ± 0.91 ng/mL; $P < 0.001$) before returning to values similar to -60 min values. Concentrations of serum cortisol were less at 0 min (7.87 ± 0.92 ng/mL) compared to -60 min values. Following administration of dextrose, there was a temperament ($P < 0.001$), time ($P < 0.001$) and temperament x time interaction ($P = 0.003$). Specifically, serum cortisol concentrations were greater ($P \leq 0.02$) in Temperamental compared to Calm steers at 45 (10.92 vs. 4.63 ± 1.08 ng/mL) and 60 min (13.20 vs. 9.5 ± 1.08 ng/mL) relative to administration of the dextrose bolus. Mean serum cortisol concentrations following administration of the dextrose bolus were greater in Temperamental (8.67 ± 0.36 ng/mL) compared to Calm steers (6.97 ± 0.35 ng/mL). Compared to 0 min values, cortisol concentrations were increased at 60 min post-dextrose administration ($P = 0.03$; 7.87 vs. 11.35 ± 0.75 ng/mL for 0 and 60 min, respectfully).

There was no effect of temperament, time or a temperament x time interaction ($P \geq 0.76$) for serum glucose prior to administration of the glucose tolerance test (Table 1). In response to administration of the dextrose bolus, serum glucose concentrations were affected by temperament ($P < 0.001$) and time ($P < 0.001$; Fig. 1A), but there was no temperament x time interaction ($P = 0.15$). In both groups of steers, serum glucose concentrations increased ($P < 0.001$) from 0 min to 10 min (78.30 vs. 196.36 ± 3.64 mg/dL) of administration of the dextrose bolus and remained elevated until 60 min (80.60 ± 3.64 mg/dL) post-challenge ($P = 0.66$; 0 min vs. 60 min). On average, mean serum glucose concentrations following dextrose administration were greater in Temperamental (115.49 ± 1.75 mg/dL) compared to Calm steers (103.51 ± 1.70 mg/dL).

Prior to dextrose administration, there was no effect of temperament, time or a temperament x time interaction for serum insulin ($P \geq 0.50$). In contrast, serum insulin concentrations were affected by temperament ($P = 0.05$) and time ($P < 0.001$; Fig. 1B) following dextrose administration, but there was no temperament x time interaction ($P = 0.48$). Regardless of temperament classification, serum insulin concentrations increased from 0 to 10 min (0.25 vs. 1.71 ± 0.09 ng/mL) after administration of dextrose ($P < 0.001$; 0 min vs. 10 min). Serum insulin concentrations returned to near baseline values within 60 min (0.34 ± 0.09 ng/mL; $P = 0.50$; 0 min vs. 60 min), similar to serum glucose concentrations. Mean serum insulin concentrations following dextrose administration were reduced in Temperamental steers (0.68 ± 0.05 ng/mL) compared to Calm steers (0.80 ± 0.05 ng/mL). There was no difference in serum glucose clearance or serum glucose half-life from 10 to 30 min or from 30 to 90 min ($P \geq 0.49$; Table 2). However, serum insulin: serum glucose was reduced in Temperamental steers overall (-60 to 150 min; $P < 0.001$) and post-challenge (0 to 150 min; $P < 0.001$; Table 2).

Prior to administration of the glucose tolerance test, there were effects of temperament and time ($P \leq 0.05$), but there was no temperament x time interaction ($P = 0.91$; Table 1) on serum NEFA concentrations. Specifically, Temperamental cattle had greater serum NEFA concentrations than Calm steers (0.30 vs. 0.20 ± 0.01 mmol/L), while NEFA concentrations increased briefly from -60 to -45 min ($P = 0.02$; 0.24 vs. 0.31 ± 0.02 mmol/L for -60 and -45 min, respectfully) before returning to baseline concentrations. After administration of the dextrose bolus, there were effects of temperament and time ($P < 0.001$), but no temperament x time interaction ($P = 0.61$) for serum NEFA concentrations. Similar to pre-challenge, Temperamental steers maintained greater serum NEFA concentrations than Calm steers (0.20 vs. 0.16 ± 0.006 mmol/L). For the time effect, serum concentrations of NEFA decreased from 0 min

to 10 min ($P = 0.01$; 0.23 vs. 0.19 ± 0.01 mmol/L) and remained below 0 min values until 90 min ($P = 0.07$; 0.20 ± 0.01), where concentrations returned to baseline values.

Serum BUN concentrations were not affected by time or a temperament x time interaction prior to the challenge ($P \geq 0.77$; Table 1); however, there was an effect of temperament ($P = 0.03$). Specifically, Calm steers had greater serum BUN concentrations than Temperamental steers (8.47 vs. 7.78 ± 0.23 mg/dL). These results were maintained following administration of dextrose, where no time or temperament x time interaction was observed ($P \geq 0.79$), but Calm steers maintained greater serum BUN concentrations than Temperamental steers ($P = 0.007$; 7.87 vs. 7.35 ± 0.13 mg/dL).

Insulin sensitivity, as calculated by RQUICKI, was affected by temperament ($P = 0.002$) but not time or a temperament x time interaction prior to the challenge ($P \geq 0.83$; Table 1). Specifically, Temperamental steers had reduced insulin sensitivity compared to Calm steers (0.50 vs. 0.57 ± 0.008 ; Fig. 2). Following administration of the challenge, there were effects of temperament and time ($P \leq 0.01$), but there was no temperament x time interaction ($P = 0.95$) on insulin sensitivity. Similar to pre-challenge, Temperamental steers had reduced insulin sensitivity compared to Calm steers (0.47 vs. 0.50 ± 0.008) after dextrose administration. Insulin sensitivity decreased from 0 min to 10 min ($P < 0.001$; 0.54 vs. 0.33 ± 0.02) and remained below 0 min values through 45 min (0.47 ± 0.02). Insulin sensitivity values then increased above 0 min values at 60 min (0.59 ± 0.02 ; $P = 0.05$) post-dextrose administration and remained elevated until 120 min (0.55 ± 0.02 ; $P = 0.50$).

3.2. Insulin sensitivity test

Prior to administration of the challenge, there was no effect of time or a temperament x time interaction ($P = 1.00$) for rectal temperature (Table 3); however, rectal temperature values were reduced ($P < 0.001$) in Temperamental steers ($38.65 \pm 0.02^{\circ}\text{C}$) compared to Calm steers ($38.72 \pm 0.02^{\circ}\text{C}$). After administration of the insulin bolus, there was no effect of time ($P = 0.63$) or a temperament x time interaction ($P = 1.00$). There was an effect of temperament ($P = 0.008$) such that Temperamental steers had reduced rectal temperature than Calm steers (38.61 vs. $38.66 \pm 0.01^{\circ}\text{C}$). Due to the temperament effect on pre-challenge rectal temperature values, rectal temperature values measured following administration of the insulin bolus were analyzed as the change in rectal temperature relative to baseline (-60 to 0 min) values. The change in rectal temperature was affected by temperament ($P < 0.001$) and time ($P < 0.001$), but there was no temperament x time interaction ($P = 0.25$; Table 3). Overall, there was less change ($P < 0.001$) in rectal temperature in Temperamental steers ($-0.03 \pm 0.01^{\circ}\text{C}$) compared to Calm steers ($-0.06 \pm 0.01^{\circ}\text{C}$). In terms of the effect of time, rectal temperature decreased below 0 min values ($0.007 \pm 0.02^{\circ}\text{C}$) at 50 min ($P = 0.02$; $-0.07 \pm 0.02^{\circ}\text{C}$) following administration of insulin and tended to remain below 0 min values until 115 min ($-0.05 \pm 0.02^{\circ}\text{C}$) post-insulin administration ($P = 0.06$).

Serum cortisol concentrations were not affected by temperament ($P = 0.91$) or by a temperament x time interaction ($P = 0.97$), but were affected by time prior to administration of insulin ($P < 0.001$; Table 3). Concentrations of serum cortisol increased briefly from -60 min to -45 min before returning to baseline values ($P = 0.02$; 10.02 vs. 12.97 ± 0.85 ng/mL). After administration of the insulin bolus, there was a temperament x time interaction ($P = 0.04$). Specifically, Calm steers had greater cortisol than Temperamental steers at 90 min ($P = 0.03$; 23.24 vs. 16.74 ± 2.06 ng/mL) and tended to at 120 min ($P = 0.08$; 16.22 vs. 11.08 ± 2.06 ng/mL), but Temperament steers had greater cortisol at 150 min than Calm steers ($P = 0.05$;

16.89 vs. 11.03 ± 2.06 ng/mL). Additionally, there was an effect of time, where serum cortisol concentrations increased 45 min after administration of insulin ($P < 0.001$; 9.28 vs. 17.67 ± 1.46 ng/mL for 0 min vs. 45 min), and remained elevated for the duration of the study ($P = 0.02$; 0 min vs. 150 min).

There were no effects of temperament, time, or a temperament x time interaction for serum glucose concentrations prior to the insulin sensitivity test ($P \geq 0.27$; Table 3).

Concentrations of serum glucose following administration of the insulin bolus were affected by temperament and time ($P \leq 0.04$; Fig. 3A), but not a temperament x time interaction ($P = 0.41$). Specifically, Calm steers had greater serum glucose than Temperamental steers (36.18 vs. 34.33 ± 0.65 mg/dL). Additionally, serum glucose concentrations began to decrease immediately after administration of insulin (78.39 vs. 62.75 ± 1.37 mg/dL for 0 min vs. 10 min; $P < 0.001$), and remained below 0 min values for the duration of the challenge. There was no effect of temperament on serum glucose clearance or serum glucose half-life from 10 to 30 min or from 30 min to 90 min ($P \geq 0.27$; Table 4).

Pre-challenge serum insulin concentrations were not affected by temperament or a temperament x time interaction ($P \geq 0.59$), but there was an effect of time ($P < 0.001$; Figure 3B). Serum insulin concentrations increased at 0 min relative to -60 min values ($P < 0.001$; 0.11 vs. 8.92 ± 1.24 ng/mL for -60 vs. 0 min). Following administration of the insulin bolus, there was a temperament x time interaction for serum insulin concentrations ($P < 0.001$; Fig. 4B). Specifically, Temperamental steers had greater ($P \leq 0.02$) serum insulin than Calm steers at 10 and 30 min after insulin administration; yet Temperamental steers had less ($P = 0.02$) serum insulin compared to Calm steers at 20 min after insulin administration. Insulin concentrations were also affected by temperament ($P < 0.001$) and time ($P < 0.001$). Serum insulin

concentrations increased 10 min after administration of the insulin bolus ($P < 0.001$; 8.92 vs. 140.59 ± 3.48 ng/mL for 0 min vs. 10 min). Serum insulin concentrations returned to 0 min values within 45 min (10.45 ± 3.48 ng/mL) post-challenge ($P = 0.76$; 0 min vs. 45 min). Post-insulin administration, Temperamental steers had greater ($P < 0.001$) serum insulin concentrations (33.21 ± 1.67 ng/mL) when compared to Calm steers (22.72 ± 1.61 ng/mL). The serum insulin: serum glucose ratio was greater ($P < 0.001$) in Temperamental compared to Calm steers both overall (-60 to 150 min) and post-challenge (0 to 150 min; Table 4).

Serum NEFA concentrations prior to the administration of the insulin challenge were not affected by time or a temperament x time interaction ($P \geq 0.23$; Table 3), but there was an effect of temperament such that Temperamental steers had greater serum NEFA than Calm steers (0.28 vs. 0.18 ± 0.01 mmol/L). After administration of the insulin bolus there were temperament and time ($P < 0.001$) effects but no temperament x time interaction ($P = 0.52$) for serum NEFA concentrations (Table 3). Throughout the post-challenge period, Temperamental steers maintained greater serum NEFA concentrations (0.28 ± 0.01 mmol/L) compared to Calm steers (0.22 ± 0.01 mmol/L). For both groups, serum concentrations of NEFA initially decreased below 0 min values (0.23 ± 0.02 mmol/L) between 20 (0.13 ± 0.02 mmol/L) to 30 (0.11 ± 0.02 mmol/L) min post-challenge ($P < 0.001$; 0 min vs. 20 min). Serum concentrations of NEFA then increased above 0 min values beginning at 60 min (0.30 ± 0.02 mmol/L) post-challenge ($P = 0.004$) and remained elevated for the duration of the challenge.

Serum concentrations of BUN were not affected by temperament, time, or a temperament x time interaction prior to administration of the insulin bolus ($P \geq 0.20$; Table 3). In contrast, the serum BUN concentration response post-challenge was affected by temperament ($P = 0.004$) and time ($P = 0.02$), but there was no temperament x time interaction ($P = 0.99$; Table 3).

Specifically, serum BUN concentrations fluctuated over time, and were reduced in Temperamental steers (7.69 ± 0.16 mg/dL) compared to Calm steers (8.28 ± 0.16 mg/dL) throughout the post-challenge period.

Prior to the administration of the insulin bolus, there was no temperament x time interaction ($P = 0.98$), but there was an effect of time for insulin sensitivity ($P < 0.001$; Table 3). Insulin sensitivity decreased at 0 min compared to -60 min values ($P < 0.001$; 0.59 vs. 0.37 ± 0.02 for -60 min vs. 0 min; Fig. 4). The decrease in insulin sensitivity at 0 min is likely due to the slight increase in insulin concentrations at 0 min (see Fig. 3B). Additionally, Temperament steers tended ($P = 0.07$) to have reduced insulin sensitivity compared to Calm steers pre-challenge (0.51 vs. 0.55 ± 0.01). After administration of the insulin bolus, there was a temperament ($P = 0.009$) and time effect ($P < 0.001$) but no temperament x time interaction ($P = 0.55$) for insulin sensitivity (Fig. 5). Mean insulin sensitivity values were reduced in Temperamental steers (0.34 ± 0.005) compared to Calm steers (0.36 ± 0.006).

4. Discussion

The glucose tolerance test tended to result in greater rectal temperatures in Calm steers compared to Temperamental steers, thus likely contributing to the greater baseline rectal temperature prior to administration of insulin. There are a limited number of studies that have measured body temperature in response to a glucose tolerance test, and this appears to be the first to measure body temperature in cattle of different temperaments in response to a glucose tolerance test. It is unclear why Temperamental steers tended to have reduced rectal temperature following administration of dextrose and prior to administration of insulin. In humans, decreased

core body temperatures have been attributed to increases in glucose [25, 26]. In dairy steers, infusion of a dextrose bolus immediately prior to administration of a lipopolysaccharide challenge **reduced the febrile response** compared to control steers [27]. Perhaps the extended elevation of glucose following administration of **dextrose** in Temperamental steers in the current study resulted in a greater decrease in rectal temperature compared to Calm cattle. However, further research is necessary to understand this response. Based on the change in rectal temperature values following the insulin sensitivity test, it appears that Temperamental steers recovered more quickly from the IST compared to Calm steers. This is supported by the decreased cortisol concentrations in Temperamental compared to Calm steers at 90 and 120 min post-challenge. These data would suggest that the insulin sensitivity test may have been more metabolically challenging in the Calm steers compared to Temperamental steers, which may be a result of the less insulin sensitive nature of the Temperamental steers.

One of the first endocrine measurements observed to differ between calm and temperamental cattle was the stress hormone cortisol [20]. While baseline values in the current study did not vary for either the glucose tolerance or insulin sensitivity tests, serum cortisol concentrations were greater in Temperamental steers following the glucose tolerance test compared to Calm steers. There is a strong relationship between cortisol and glucose such that elevation of cortisol increases glycogenolysis and gluconeogenesis, resulting in increased circulating glucose concentrations [28]. **A greater decrease in serum cortisol was observed in Calm steers than Temperamental steers in response to increasing serum glucose, further demonstrating alterations in the HPA-axis and metabolic systems in these cattle.** Further, studies conducted in the late 1930's reported that removal of the adrenal gland caused a dramatic decrease in glucose concentrations, thus the association of glucocorticoids (i.e., cortisol) with

glucose regulation [28]. Therefore, the greater serum cortisol response in the Temperamental steers compared to the Temperamental Calm steers is not unexpected.

Serum glucose concentrations were greater in Temperamental steers when compared to Calm steers in response to administration of dextrose. This may be the result of decreased rate of uptake or disposal of glucose by Temperamental steers, or an increase in glucose production [16]; however, the former is more probable. Serum insulin concentrations were reduced in Temperamental steers in response to dextrose administration compared to Calm steers. Thus, it is possible that the lower insulin concentrations observed in response to dextrose administration contributed to the elevation in glucose concentrations in Temperamental steers compared to Calm steers. Additionally, Temperamental steers had greater body weights prior to and following the study. Greater body weight in the Temperamental steers may have influenced the serum glucose response. However, baseline serum glucose concentrations did not differ between temperament groups which suggest that body weight did not significantly affect the glucose response to dextrose administration, which may be influenced to a greater magnitude by differences in insulin concentrations during this study.

Different serum insulin responses were observed between the glucose tolerance and insulin sensitivity tests, with the insulin sensitivity test producing a greater serum insulin response in Temperamental steers than Calm steers. The greater insulin concentrations observed in Temperamental steers following administration of the insulin bolus may be caused by decreases in insulin extraction. Impaired insulin extraction has been demonstrated *in vivo* in dogs with elevated free fatty acids at high and low insulin concentrations [29], analogous to the insulin and NEFA concentrations observed in the current study. Similar observations were made using *in situ* experiments of free fatty acid perfused rat livers (major organ responsible for insulin

clearance) [30]. Interestingly, changes in glucose perfusion concentrations did not alter insulin extraction [30]. Further, Svedberg, et al. [31] reported reduced binding, action, and degradation of insulin when fatty acids were elevated in cultures with rat hepatocytes. In models of obesity combined with diabetes in humans, and in the early lactation dairy cow, elevated free fatty acids are typically observed along with the hyperinsulinemia [32, 33]. However, this is not the case in temperamental cattle as no differences in basal serum insulin secretion have been observed compared to calm cattle in this study and others [5]. Further, in response to an LPS challenge, which typically results in release of glucose, insulin, and NEFAs, both serum glucose and insulin were reduced in temperamental compared to calm bulls, while serum NEFAs were greater [5]. Therefore, Temperamental cattle may differ from human models of diabetes and the early lactation dairy cow such that elevated NEFAs are observed in the absence of hyperinsulinemia and hyperglycemia. Additionally, elevated NEFAs can mediate circulating glucose concentrations by decreasing the GLUT4 receptor, thus altering insulin-mediated glucose uptake [14, 33]. Also, Administration of nicotinic acid to Holstein cows resulted in an increase in glucose clearance following a glucose tolerance test, which may have been a result of increased responsiveness to insulin due to a reduction in serum NEFA concentrations [34].

Insulin sensitivity, as demonstrated through calculation of the RQUICKI, was decreased throughout the glucose tolerance and insulin sensitivity tests in Temperamental cattle. The RQUICKI calculation used glucose, insulin and NEFA concentrations to determine an index of insulin sensitivity. These calculations support the differences observed in serum glucose and serum insulin in response to the two challenges, where there appeared to be disassociation between serum glucose and insulin concentrations. One common theme across the two challenges was the greater serum NEFA concentrations in Temperamental compared to Calm

steers. Elevated serum NEFA concentrations have been demonstrated to alter the sensitivity of dairy cows to insulin [16], similar to what is observed in the current study in Temperamental beef steers. Acute increases in free fatty acids can stimulate insulin secretion [29]. However, it is likely that the extended and “chronic” elevation of serum NEFAs results in a down-regulation of glucose-stimulated insulin secretion in Temperamental cattle via mechanisms associated with insulin insensitivity, as observed in the decreased serum insulin response to the glucose tolerance test. There is evidence that elevated NEFA concentrations can directly affect pancreatic beta cells, and may lead to apoptosis [15], while changes in the lipid content of cells, particularly in the pancreas or liver, can affect intracellular signaling and ligand recognition, which may lead to differential cellular responses [32]. Thus, there appears to be a significant role of elevated NEFA concentrations on modulating insulin sensitivity in cattle and other species.

It is unclear whether greater serum NEFA concentrations are a result of insulin insensitivity or whether the elevated serum NEFA concentrations are resulting in insulin insensitivity. Hayirli [16] described three mechanisms for insulin insensitivity: 1) insensitivity at the pre-receptor level (i.e., degradation of insulin or a decrease in insulin production), 2) insensitivity at the receptor level (i.e., poor binding), or 3) insensitivity at the post-receptor level (i.e., poor transduction of the signal from the insulin receptor). Based on the data from the current study, it is possible that there is an alteration at both the pre- and post-receptor levels. Increasing serum NEFA concentrations, via a lipid emulsion, increased serum glucose concentrations in Holstein steers following a LPS challenge compared to Control steers, which supports the implication that elevated NEFAs may be causing alterations in insulin sensitivity [27]. However, there is limited literature support utilizing livestock models.

The elevated serum NEFA and decreased serum BUN concentrations observed in the Temperamental steers in this study are similar to previous reports [5, 7]. Also, the serum concentrations of NEFA exhibited by the Temperamental cattle are similar to what have been observed in transition dairy cows [35, 36]. Decreases in serum BUN in Temperamental steers may be indicative of increased nitrogen retention compared to Calm steers, likely a result of a reduction in proteolysis or an increase in protein synthesis [37]. Lipid accumulation in the liver has been demonstrated to decrease ureagenesis in bovine hepatocytes [38]. Also, increases in growth hormone (GH) can cause increases in lipolysis and protein synthesis, thus increasing nitrogen retention [39]. Growth hormone has been demonstrated to have a role in regulating metabolism through decreasing glucose uptake and utilization and increasing free fatty acids. However, measurement of circulating GH concentrations is necessary to confirm a role of GH in altered metabolic functions in temperamental versus calm cattle.

5. Conclusion

These data demonstrate that differences exist in the manner that Temperamental steers respond to glucose and insulin. The altered response to glucose and insulin observed in Temperamental steers, possibly may result in differences in the utilization of energy sources or in energy redistribution when compared to Calm cattle. It appears this may be mediated by elevated serum NEFA concentrations and decreased insulin extraction. These data further implicate metabolic differences as a primary factor associated with altered immune function and performance traits observed between Calm and Temperamental cattle. Further research is necessary to determine the mechanisms that ultimately result in elevated serum NEFA

494 concentrations and decreased insulin sensitivity in Temperamental cattle in order to more fully
495 elucidate the effect of temperament on metabolism.

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References

- [1] Fordyce, G, Dodt, RM, Wythes, JR. Cattle Temperaments in Extensive Beef Herds in Northern Queensland .1. Factors Affecting Temperament. Australian Journal of Experimental Agriculture. 1988;28:683-687.
- [2] Burdick, NC, Carroll, JA, Hulbert, LE, Dailey, JW, Ballou, MA, Randel, RD, Willard, ST, Vann, RC, Welsh, TH. Temperament influences endotoxin-induced changes in rectal temperature, sickness behavior, and plasma epinephrine concentrations in bulls. Innate Immunity. 2011;17:355-364.
- [3] Burdick, N, Randel, R, Carroll, J, Welsh, T. Interactions between temperament, stress, and immune function in cattle. International Journal of Zoology. 2011;2011:
- [4] Burdick, NC, Carroll, JA, Randel, RD, Willard, ST, Vann, RC, Chase, CC, Lawhon, SD, Hulbert, LE, Welsh, TH. Influence of temperament and transportation on physiological and endocrinological parameters in bulls. Livestock Science. 2011;139:213-221.
- [5] Burdick Sanchez, NC, Carroll, JA, Randel, RD, Vann, RC, Welsh, TH. Associations between endotoxin-induced metabolic changes and temperament in Brahman bulls. Journal of Animal Physiology and Animal Nutrition. 2014;98:178-190.
- [6] Curley, KO, Jr., Neuendorff, DA, Lewis, AW, Cleere, JJ, Welsh, TH, Jr., Randel, RD. Functional characteristics of the bovine hypothalamic-pituitary-adrenal axis vary with temperament. Horm Behav. 2008;53:20-7.
- [7] Cafe, LM, Robinson, DL, Ferguson, DM, Geesink, GH, Greenwood, PL. Temperament and hypothalamic-pituitary-adrenal axis function are related and combine to affect growth, efficiency, carcass, and meat quality traits in Brahman steers. Domestic Animal Endocrinology. 2011;40:230-240.

523 [8] Carroll, JA, Burdick Sanchez, NC, Hulbert, LE, Ballou, MA, Dailey, JW, Caldwell, LC,
524 Vann, RC, Welsh, TH, Jr., Randel, RD. Sexually dimorphic innate immunological responses of
525 pre-pubertal Brahman cattle following an intravenous lipopolysaccharide challenge. *Vet*
526 *Immunol Immunopathol.* 2015;166:108-15.

527 [9] Oliphint, RA. Evaluation of the inter-relationships of temperament, stress responsiveness and
528 immune function in beef calves. M.S. thesis, Texas A&M University, College Station. 2006;

529 [10] Fell, LR, Colditz, IG, Walker, KH, Watson, DL. Associations between temperament,
530 performance and immune function in cattle entering a commercial feedlot. *Australian Journal of*
531 *Experimental Agriculture.* 1999;39:795-802.

532 [11] Fordyce, G, Wythes, JR, Shorthose, WR, Underwood, DW, Shepherd, RK. Cattle
533 Temperaments in Extensive Beef Herds in Northern Queensland .2. Effect of Temperament on
534 Carcass and Meat Quality. *Australian Journal of Experimental Agriculture.* 1988;28:689-693.

535 [12] Burrow, HM, Dillon, RD. Relationships between temperament and growth in a feedlot and
536 commercial carcass traits of *Bos indicus* crossbreds. *Australian Journal of Experimental*
537 *Agriculture.* 1997;37:407-411.

538 [13] Muller, R, von Keyserlingk, MAG. Consistency of flight speed and its correlation to
539 productivity and to personality in *Bos taurus* beef cattle. *Applied Animal Behaviour Science.*
540 2006;99:193-204.

541 [14] Mohamed-Ali, V, Pinkney, JH, Coppack, SW. Adipose tissue as an endocrine and paracrine
542 organ. *International Journal of Obesity.* 1998;22:1145-1158.

543 [15] Contreras, GA, Sordillo, LM. Lipid mobilization and inflammatory responses during the
544 transition period of dairy cows. *Comparative Immunology Microbiology and Infectious*
545 *Diseases.* 2011;34:281-289.

546 [16] Hayirli, A. The role of exogenous insulin in the complex of hepatic lipidosis and ketosis
547 associated with insulin resistance phenomenon in postpartum dairy cattle. *Veterinary Research*
548 *Communications*. 2006;30:749-774.

549 [17] Thiebaud, D, Jacot, E, DeFronzo, RA, Maeder, E, Jequier, EFelber, JP. The effect of graded
550 doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. *Diabetes*.
551 1982;31:957-63.

552 [18] King, DA, Pfeiffer, CES, Randel, RD, Welsh, TH, Oliphint, RA, Baird, BE, Curley, KO,
553 Vann, RC, Hale, DS, Savell, JW. Influence of animal temperament and stress responsiveness on
554 the carcass quality and beef tenderness of feedlot cattle. *Meat Science*. 2006;74:546-556.

555 [19] Reuter, RR, Carroll, JA, Hulbert, LE, Dailey, JW, Galyean, ML. Technical note:
556 Development of a self-contained, indwelling rectal temperature probe for cattle research. *J Anim*
557 *Sci*. 2010;88:3291-5.

558 [20] Curley, KO, Jr., Paschal, JC, Welsh, TH, Jr., Randel, RD. Technical note: Exit velocity as a
559 measure of cattle temperament is repeatable and associated with serum concentration of cortisol
560 in Brahman bulls. *J Anim Sci*. 2006;84:3100-3.

561 [21] Burrow, HM, Seifert, GW, Corbet, NJ. A new technique for measuring temperament in
562 cattle. *Proceedings of the Australian Society of Animal Production*. 1988;17:154-157.

563 [22] Hammond, AC, Olson, TA, Chase, CC, Jr., Bowers, EJ, Randel, RD, Murphy, CN, Vogt,
564 DW, Tewolde, A. Heat tolerance in two tropically adapted *Bos taurus* breeds, Senepol and
565 Romosinuano, compared with Brahman, Angus, and Hereford cattle in Florida. *J Anim Sci*.
566 1996;74:295-303.

567 [23] Bernhard, BC, Burdick, NC, Rathmann, RJ, Carroll, JA, Finck, DN, Jennings, MA, Young,
568 TR, Johnson, BJ. Chromium supplementation alters both glucose and lipid metabolism in feedlot
569 cattle during the receiving period. *Journal of Animal Science*. 2012;90:4857-4865.

570 [24] Holtenius, P, Holtenius, K. A model to estimate insulin sensitivity in dairy cows. *Acta*
571 *Veterinaria Scandinavica*. 2007;49:29.

572 [25] Green, JH, Macdonald, IA. The Influence of Intravenous Glucose on Body-Temperature.
573 *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences*. 1981;66:465-473.

574 [26] Fiorentini, A, Muller, EE. Sensitivity of Central Chemoreceptors Controlling Blood-
575 Glucose and Body-Temperature during Glucose Deprivation. *Journal of Physiology-London*.
576 1975;248:247-271.

577 [27] Burdick Sanchez, NC, Carroll, JA, Donaldson, JR, Buntyn, JO, Schmidt, TB. Exogenous
578 administration of lipids to steers alters aspects of the innate immune response to endotoxin
579 challenge. *Innate Immun*. 2015;21:512-522.

580 [28] Long, CNH, Katzin, B, Fry, EG. The adrenal cortex and carbohydrate metabolism.
581 *Endocrinology*. 1940;26:309-344.

582 [29] Wiesenthal, SR, Sandhu, H, McCall, RH, Tchipashvili, V, Yoshii, H, Polonsky, K, Shi, ZQ,
583 Lewis, GF, Mari, AGiacca, A. Free fatty acids impair hepatic insulin extraction in vivo (vol 48,
584 766, 1999). *Diabetes*. 1999;48:1348-1348.

585 [30] Svedberg, J, Stromblad, G, Wirth, A, Smith, UBjorntorp, P. Fatty-Acids in the Portal-Vein
586 of the Rat Regulate Hepatic Insulin-Clearance. *Journal of Clinical Investigation*. 1991;88:2054-
587 2058.

588 [31] Svedberg, J, Bjorntorp, P, Smith, U, Lonnroth, P. Free-fatty acid inhibition of insulin
589 binding, degradation, and action in isolated rat hepatocytes. *Diabetes*. 1990;39:570-4.

590 [32] Sordillo, LM, Contreras, GA, Aitken, SL. Metabolic factors affecting the inflammatory
591 response of periparturient dairy cows. *Anim Health Res Rev.* 2009;10:53-63.

592 [33] Iozzo, P, Lautamaki, R, Geisler, F, Virtanen, KA, Oikonen, V, Haaparanta, M, Yki-
593 Jarvinen, H, Ferrannini, E, Knuuti, J, Nuutila, P. Non-esterified fatty acids impair insulin-
594 mediated glucose uptake and disposition in the liver. *Diabetologia.* 2004;47:1149-1156.

595 [34] Pires, JA, Pescara, JB, Grummer, RR. Reduction of plasma NEFA concentration by
596 nicotinic acid enhances the response to insulin in feed-restricted Holstein cows. *J Dairy Sci.*
597 2007;90:4635-42.

598 [35] Busato, A, Faissler, D, Kupfer, U, Blum, JW. Body condition scores in dairy cows:
599 Associations with metabolic and endocrine changes in healthy dairy cows. *Journal of Veterinary*
600 *Medicine Series a-Physiology Pathology Clinical Medicine.* 2002;49:455-460.

601 [36] Accorsi, PA, Govoni, N, Gaiani, R, Pezzi, C, Seren, E, Tamanini, C. Leptin, GH, PRL,
602 insulin and metabolic parameters throughout the dry period and lactation in dairy cows.
603 *Reproduction in Domestic Animals.* 2005;40:217-223.

604 [37] Lang, CH. Elevated plasma free fatty acids decrease basal protein synthesis, but not the
605 anabolic effect of leucine, in skeletal muscle. *American Journal of Physiology-Endocrinology*
606 *and Metabolism.* 2006;291:E666-E674.

607 [38] Strang, BD, Bertics, SJ, Grummer, RR, Armentano, LE. Effect of long-chain fatty acids on
608 triglyceride accumulation, gluconeogenesis, and ureagenesis in bovine hepatocytes. *Journal of*
609 *Dairy Science.* 1998;81:728-739.

610 [39] Meier, S, Gore, PJS, Bamett, CME, Cursons, RT, Phipps, DE, Watkins, KA, Verkerk, GA.
611 Metabolic adaptations associated with irreversible glucose loss are different to those observed
612 during under-nutrition. *Domestic Animal Endocrinology.* 2008;34:269-277.

613 **Tables**

614 **Table 1** Effect of temperament on rectal temperature and serum cortisol, glucose, insulin, non-esterified fatty acids and blood urea
615 nitrogen and insulin sensitivity after an intravenous glucose tolerance test in beef steers.

Variable	Period ¹	Temperament		SEM	P-value		
		Calm	Temperamental		Temperament	Time	Temperament x Time
Rectal temperature, °C	Pre	38.95	38.94	0.02	0.84	0.82	0.99
	Post	38.83	38.80	0.01	0.10	0.001	1.00
Cortisol, ng/mL	Pre	10.24	11.11	0.58	0.29	<0.001	0.56
	Post	6.97	8.67	0.36	<0.001	<0.001	0.003
Glucose, mg/dL	Pre	77.76	80.14	1.51	0.29	0.85	0.76
	Post	103.51	115.49	1.75	<0.001	<0.001	0.15
Insulin, ng/mL	Pre	0.23	0.21	0.02	0.50	0.58	0.66
	Post	0.80	0.68	0.05	0.05	<0.001	0.48
Non-esterified fatty acids, mmol/L	Pre	0.20	0.30	0.01	<0.001	0.04	0.91
	Post	0.16	0.20	0.01	<0.001	<0.001	0.61
Blood urea nitrogen, mg/dL	Pre	8.47	7.78	0.23	0.03	0.77	0.96
	Post	7.87	7.35	0.13	0.01	0.79	0.99
Insulin Sensitivity	Pre	0.57	0.50	0.02	0.002	0.83	0.92
	Post	0.50	0.47	0.01	0.01	<0.001	0.95

616 ¹Period here refers to Pre- and Post- glucose tolerance test. Specifically, Pre is the average for -60 to 0 min, and Post is the average for
617 0 to 150 min relative to administration of glucose at 0 min.

Table 2 Effect of temperament on glucose and insulin kinetics after an intravenous glucose tolerance test in beef steers.

Variable	Temperament		SEM	P-value
	Calm	Temperamental		
Glucose clearance rate, %/min				
10 to 30 min	3.09	2.73	0.39	0.48
30 to 90 min	0.40	0.64	0.20	0.29
Glucose half-life, min				
10 to 30 min	24.67	26.76	4.08	0.49
30 to 90 min	187.43	118.70	43.32	0.19
Serum insulin (ng/mL): serum glucose (mg/dL)				
-60 to 150 min	0.054	0.0042	0.00025	< 0.001
0 to 150 min	0.066	0.0500	0.00034	0.001

622 **Table 3** Effect of temperament on rectal temperature and serum cortisol, glucose, insulin, non-esterified fatty acids and blood urea
623 nitrogen and insulin sensitivity after an intravenous insulin sensitivity test in beef steers.

Variable	Period ¹	Temperament		SEM	P-value		
		Calm	Temperamental		Temperament	Time	Temperament x Time
Rectal temperature, °C	Pre	38.72	38.65	0.02	0.01	1.00	1.00
	Post	38.66	38.61	0.01	0.01	0.63	1.00
	Change ²	-0.06	-0.03	0.01	<0.001	<0.001	0.25
Cortisol, ng/mL	Pre	9.93	9.84	0.54	0.91	0.001	0.97
	Post	14.43	14.03	0.69	0.67	<0.001	0.04
Glucose, mg/dL	Pre	76.42	76.53	1.0	0.94	<0.001	0.30
	Post	36.18	34.32	0.65	0.04	<0.01	0.41
Insulin, ng/mL	Pre	1.59	2.19	0.80	0.59	<0.001	0.85
	Post	22.72	33.21	1.67	<0.001	<0.001	<0.001
Non-esterified fatty acids, mmol/L	Pre	0.18	0.28	0.01	<0.001	0.23	0.94
	Post	0.22	0.28	0.01	<0.001	<0.001	0.52
Blood urea nitrogen, mg/dL	Pre	7.69	7.34	0.19	0.20	0.31	0.97
	Post	8.26	7.70	0.15	0.01	0.15	0.99
Insulin Sensitivity	Pre	0.55	0.51	0.01	0.07	<0.001	0.98
	Post	0.36	0.34	0.01	0.01	<0.001	0.55

624 ¹Period here refers to Pre- and Post- Glucose Tolerance Test. Specifically, Pre is the average for -60 to 0 min, and Post is the average
625 for 0 to 150 min relative to administration of insulin at 0 min.

626 ²Change refers to the average values for the change in rectal temperature relative to average Pre-challenge values.

Table 4 Effect of temperament on glucose and insulin kinetics after an intravenous insulin sensitivity test in beef steers.

	Temperament			
Variable	Calm	Temperamental	SEM	P-value
Glucose clearance rate, %/min				
10 to 30 min	2.94	3.86	0.68	0.25
30 to 90 min	4.70	4.87	0.71	0.85
Glucose half-life, min				
10 to 30 min	22.21	18.53	2.85	0.27
30 to 90 min	18.45	23.63	5.59	0.50
Serum insulin (ng/mL): serum glucose mg/dL)				
-60 to 150 min	0.34	0.52	0.03	< 0.001
0 to 150 min	0.50	0.76	0.04	< 0.001

Figure legends

Fig. 1. Effect of temperament on the serum (A) glucose and (B) insulin response in Angus-cross steers in response to a glucose tolerance test. Angus-cross steers (n = 16 Calm and 15 Temperamental) were selected based on Temperament Score measured at weaning. Blood samples were collected at -60 (0800 h), -45, -30, -15, 0, 10, 20, 30, 45, 60, 90, 120, and 150 min (1130 h) relative to i.v. administration of 50% dextrose solution (0.5 mL/kg BW) at 0 min. Data presented as LSM $\pm$ SEM. (A) There was no effect of temperament, time or temperament x time interaction ($P \geq 0.76$) for serum glucose prior to administration of the dextrose bolus. Following dextrose administration, serum glucose concentrations were affected by temperament and time ($P < 0.001$) but there was no temperament x time interaction ($P = 0.15$); (B) Serum insulin concentrations were not affected by temperament, time or a temperament x time interaction ($P \geq 0.50$) prior to dextrose challenge. Following administration of the dextrose bolus, serum insulin concentrations were affected by temperament ($P = 0.05$) and time ($P < 0.001$), but not by a temperament x time interaction ($P = 0.48$).

Fig. 2. Effect of temperament on the insulin sensitivity response in Angus-cross steers in response to a glucose tolerance test. Angus-cross steers (n = 16 Calm and 15 Temperamental) were selected based on Temperament Score measured at weaning. Blood samples were collected at -60 (0800 h), -45, -30, -15, 0, 10, 20, 30, 45, 60, 90, 120, and 150 min (1130 h) relative to i.v. administration of 50% dextrose solution (0.5 mL/kg BW) at 0 min. Insulin sensitivity was calculated based on the Revised Quantitative Insulin Sensitivity Check Index (RUIICKI) as previously described [24]. Data presented as LSM $\pm$ SEM. Insulin sensitivity was affected by temperament ($P = 0.002$) but not time or temperament x time ($P \geq 0.83$) prior to administration

of dextrose. Following the dextrose bolus administration, insulin sensitivity was affected by temperament and time ($P \leq 0.01$) but not temperament x time ($P = 0.95$).

Fig. 3. Effect of temperament on the serum (A) glucose and (B) insulin response in Angus-cross steers in response to an insulin sensitivity test. Angus-cross steers (n = 16 Calm and 15 Temperamental) were selected based on Temperament Score measured at weaning. Blood samples were collected at -60 (1300 h), -45, -30, -15, 0, 10, 20, 30, 45, 60, 90, 120, and 150 min (1630 h) relative to i.v. administration of bovine insulin (2.5 IU/kg BW) at 0 min. Data presented as LSM $\pm$ SEM. (A) Concentrations of serum glucose were not affected by temperament, time or temperament x time prior to insulin administration ($P \geq 0.27$). Following insulin administration, serum glucose was affected by temperament and time ($P \leq 0.04$) but there was no temperament x time interaction ($P = 0.41$); (B) Serum insulin concentrations prior to insulin administration were not affected by temperament, time or temperament x time ($P \geq 0.59$). Following insulin administration, serum insulin concentrations were affected by temperament, time and a temperament x time interaction ($P < 0.001$). *Temperaments at the same time point differ by $P \leq 0.005$.

Fig. 4. Effect of temperament on the insulin sensitivity response in Angus-cross steers in response to an insulin sensitivity test. Angus-cross steers (n = 16 Calm and 15 Temperamental) were selected based on Temperament Score measured at weaning. Blood samples were collected at -60 (1300 h), -45, -30, -15, 0, 10, 20, 30, 45, 60, 90, 120, and 150 min (1630 h) relative to i.v. administration of bovine insulin (2.5 IU/kg BW) at 0 min. Insulin sensitivity was calculated based on the Revised Quantitative Insulin Sensitivity Check Index (RUIICKI) as previously described [24]. Data presented as LSM $\pm$ SEM. Prior to insulin administration, there was a time effect ($P < 0.001$) and a tendency for a temperament effect ($P = 0.07$), but there was no

676 temperament x time interaction ($P = 0.98$) for insulin sensitivity. Following administration of the
677 insulin bolus, insulin sensitivity was affected by temperament ($P = 0.009$) and time ($P < 0.001$),
678 but not temperament x time ($P = 0.55$).

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Fig. 1

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