

Blood lactate and rectal temperature can predict exit velocity of beef feedlot steers

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ABSTRACT: Interest in beef cattle temperament has increased due to growing consumer awareness of animal welfare and increased concern for handler safety. Temperament measures are based on behavioral responses to a perceived stressor. Subjective chute scoring has been used to give a numeric value to temperament; however, the subjectivity and variability among observers have been questioned. To deal with the perceived subjectivity and variability, other researchers have used exit velocity. Researchers have related faster exit velocities to increased cortisol and plasma lactate. The objectives of this study were to compare temperament between feedlot steers and heifers and to confirm chute side measures of temperament relationship to physiological responses to stress. Body temperature, blood and plasma lactate, serum glucose, salivary and serum cortisol concentrations were measured on *Bos taurus* commercial crossbred feedlot cattle ($n = 197$). Fast, medium, and slow classifications were developed from exit velocities. Plasma lactate was significantly different between all exit velocity classes. Exit velocity and physiological measures indicated that heifers were more excitable (faster exit velocities ($P = 0.003$),

higher plasma lactate concentrations ($P = 0.03$), and cortisol concentrations ($P = 0.001$)). Simple correlations among these variables indicated body temperature (heifers $r = 0.44$, $P < 0.0001$; steers $r = 0.45$, $P < .0001$), plasma lactate (heifers $r = 0.52$, $P < 0.0001$; steers $r = 0.63$, $P < 0.0001$), blood lactate (heifers $r = 0.53$, $P < 0.001$; steers $r = 0.59$, $P < 0.001$), and glucose (heifers $r = 0.54$, $P < 0.001$; steers $r = 0.32$, $P < 0.003$) were all related to exit velocity. Cortisol measures were not correlated to exit velocity in steers but were in heifers. Linear models constructed and evaluated using the Akaike information criterion indicated that blood lactate in combination with rectal temperature were strong candidates to predict exit velocity. Using the discriminate function analysis, the model correctly categorized fast and slow classifications 69.23% and 61.54%, respectively, indicating that in combination measures of body temperature and blood lactate can potentially increase accuracy of temperament identification or replace exit velocity as a measure of temperament. The plasma lactate and rectal temperature have the potential to become strong objective measures to augment or replace exit velocity.

Key words: beef cattle, cortisol, exit velocity, lactate, temperament

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INTRODUCTION

Consumers as well as producers are concerned with animal welfare. Temperament and coping styles of an animal have been linked to

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welfare in livestock species (Finkemeier et al., 2018). Temperament of livestock has been evaluated using a variety of tests including observational tests such as docility scores or more objective measures such as exit velocity. Results from numerous studies, indicate cattle with excitable temperaments, based on chute score or exit velocity, have decreased average daily gains (ADGs) with lower carcass weights (Ferguson et al., 2006; Café et al., 2011; Turner et al., 2011; Francisco et al., 2012; Braga et al., 2018). Methods to identify more temperamental animals can lead to improvement in welfare and production efficiency. Currently, exit velocity is recognized as the most practical objective measure for assessing temperament (Café et al., 2011). However, few producers would be willing to purchase the equipment necessary for measuring exit velocity. Chute scores have commonly been used but due to the subjectivity and associated variability among researchers, chute scores have been questioned for repeatability and consistency as a measure of temperament. Increased stress response in more excitable animals can cause muscle metabolic pathways to switch from aerobic to anaerobic metabolism leading to increased lactate production. Most lactate is transported out of the muscle into the blood via the monocarboxylate transporters MCT1 and MCT4 (Coles et al., 2004; Baird et al., 2007; Thomas et al., 2012) increasing blood lactate concentrations in the blood (Holmes et al., 1972). Boles et al., (2015) found blood lactate to be significantly correlated with chute scores and exit velocity. Identifying easy chute side measurements of temperament could allow for identification of temperamental animals enabling altered handling to improve welfare of animals as well as improving production (Hall et al., 2011; Cooke, 2014). Therefore, the purpose of this study was to evaluate the relationship between blood lactate measures and physiological biomarkers as a proxy for exit velocity to enable a more objective and practical measure of temperament.

MATERIALS AND METHODS

Cattle Selection and Management

Data were collected in compliance with Montana State University Agriculture Animal Care and Use Committee under the Animal Care approval number 2014-AA09. A total of 197 *Bos taurus* commercial crossbred feedlot cattle were sampled from a commercial, certified Beef Quality Assurance feedlot in Chappell Nebraska. Pens were

selected by feedlot personnel to achieve a range of temperament with similar days on feed. Animals were fed a standard concentrate feedlot diet, consisting of 94% concentrate composed of rolled corn, beet pulp, dried distillers grains, protein supplement, corn silage and ground hay (dry matter basis 14.58% crude protein and 0.61 Mcal/lb net energy for gain). Animals were fed three times daily. All animals used in the study received diets that contained melengestrol acetate (MGA), 0.5 mg/day. Chute side and blood draw were measured on 3 consecutive days in March with the first animal being handled between 8:10 a.m. and 8:20 a.m. The last animal was handled on each day before 10:30 a.m. Animals were brought in two groups each day with all animals from one group being completed before animals from the second group were moved to the handling facility. Movement of animals to and from pens as well as chute control was conducted by feedlot personnel.

Temperament Measures

The feedlot operator was requested to select pens of cattle with extremes in temperament to increase the variation for sampling. A Polaris timer system (Farmtek Inc., Wylie, TX) was used to measure exit velocity with photo-transmitters placed 1.83 and 3.65 m in front of chute. Chute scores as described in Table 1 were assigned by the same individual for all animal (BIF, 2018). Chute scores were assigned according to behavior throughout handling process, i.e., entering hydraulic squeeze chute, while measures were being collected, and exit from the squeeze chute.

Chute Side Physiology Measures

Rectal temperature was taken using a Jorgensen Laboratories Digital Thermometer fitted with a rectal probe (Jorgensen Laboratories, Loveland, CO). Lactate was determined on < 2 μ L of whole blood from jugular venipuncture using a Lactate Pro meter (Akray Inc., Minami-ku, Kyoto, Japan) and reported as millimolar concentration.

Blood Collection and Preparation

Blood samples were drawn from the jugular vein using 1.25 mm \times 30 mm Vacuette needles (Greiner Bio-One, Kremst nster, Austria) whereas the animals were restrained in a hydraulic squeeze chute (Moly Manufacturing, Lorraine, KS). Two different Vacutainer tubes (Becton, Dickinson and Company,

Table 1. Chute score as recommended by Beef Improvement Federation

Score	Description
1	Docile Mild disposition. Gentle and easily handled. Stands and moves slowly during processing. Undisturbed, settled, somewhat dull. Does not pull on headgate when in chute. Exits chute calmly.
2	Restless Quieter than average, but may be stubborn during processing. May try to back out of chute or pull back on headgate. Some flicking of tail. Exits chute promptly.
3	Nervous Typical temperament is manageable, but nervous and impatient. A moderate amount of struggling, movement and tail flicking. Repeated pushing and pulling on headgate. Exits chute briskly.
4	Flighty (Wild) Jumpy and out of control, quivers and struggles violently. May bellow and froth at the mouth. Continuous tail flicking. Defecates and urinates during processing. Frantically runs fence line and may jump when penned individually. Exhibits long flight distance and exits chute wildly.
5	Aggressive May be similar to Score 4, but with added aggressive behavior, fearfulness, extreme agitation, and continuous movement which may include jumping and bellowing while in chute. Exits chute frantically and may exhibit attack behavior when handled alone.
6	Very Aggressive Extremely aggressive temperament. Thrashes about or attacks wildly when confined in small, tight places. Pronounced attack behavior.

<https://beefimprovement.org/library-2/bif-guidelines>.

Franklin Lakes, NJ) were used for blood collection, blood for the purpose of measuring lactate and cortisol were collected in silicone-coated tubes (10-mL blood), and sodium fluoride potassium oxalate coated tubes were used for collecting blood to measure glucose (4-mL blood). Two saliva samples were collected from each animal using Salivette tubes (Sarstedt AG) by massaging the glands to activate saliva production. Salivette tubes were kept on ice during collection and were placed on dry ice (about -79°C) for transportation back to Montana State University. The sodium fluoride potassium oxalate Vacutainer tubes were stored on ice during collection and were centrifuged at $1,177 \times g$ for 30 min using a Cole-Parmer VS-3400 centrifuge (Vernon-Hills, IL). Blood samples were centrifuged within 4 h from collection to prevent enzymatic breakdown of glucose (Mikesh and Bruns, 2008). After samples were centrifuged, serum was decanted from cellular components and stored in 8 mL Nalgene General Long-Term Storage Cryogenic Tubes (Thermo Fisher Scientific Inc., Waltham, NC) and stored at -20°C until analyzed. Silicone-coated serum Vacutainer tubes were used to collect blood for lactate, and cortisol measurements. Vacutainer tubes were stored on ice during collection and stored at 1°C for 24 to 36 h after collection. Serum tubes were centrifuged either using Thermo Scientific Sorvall Legend RT+ or Cole-Parmer VS-3400 centrifuge at $1,177 \times g$ for 30 min. After samples were centrifuged, serum was decanted from cellular components and stored in 8 mL Nalgene General Long-Term Storage Cryogenic Tubes and stored at -80°C until analyzed. Salivette tubes were thawed for 20 min at 21°C . To recover saliva, Salivette tubes were placed in a Thermo Scientific Sorvall Legend RT+ centrifuge and were centrifuged for 4 min at

$1,000 \times g$ at 8°C . Once saliva was separated from internal cotton swab, the swabs were discarded and the saliva for each animal was combined into one tube. After combining, samples were stored at -20°C until analyzed.

Glucose

Infinity Glucose (lot # 241531; Thermo Scientific) microplate assay procedure was used to analyze serum concentration of glucose. Serum was brought to room temperature and samples were assayed in duplicate. Means, standard deviations, and the coefficient of variation were calculated for all samples. Any sample with a coefficient of variation greater than 12% was repeated. Intra- and inter-coefficients of variation for bovine serum containing 173.67 mg/dL of glucose was 6.02% and 5.4%, respectively, and for serum containing 176.74 mg/dL glucose were 7.25% and 4.4%, respectively.

Serum Lactate

Serum Lactate Assay Kit II (Biovision Inc, Milpitas, CA) microplate assay procedure was used to analyze serum concentration of lactate. Frozen serum samples were placed in a tepid water bath to bring samples to room temperature before analysis. The following equation was used to report actual concentrations of lactate: $\text{nmol}/5 \mu\text{L} \times \text{Dilution Factor} = \text{nmol}/\mu\text{L}$. Means, standard deviations, and the coefficient of variation were calculated for all samples. Any sample with a coefficient of variation greater than 12% was repeated. The intra- and inter-coefficient of variation for serum lactate for bovine serum containing 3.5 mM was 6.14%

and 14.8%, respectively, and for serum containing 0.78 mM was 10.2% and 17.9%, respectively.

Cortisol

An Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit from Salimetrics (Salimetrics, State College, PA) was used to analyze salivary cortisol concentrations. Standards, controls, and salivary samples were assayed in triplicate. Means, standard deviations, and the coefficient of variation were calculated for all samples. Any sample with a coefficient of variation greater than 12% was repeated. The intra- and inter-assay coefficients of variation for salivary cortisol for pooled bovine saliva containing 0.12 µg/dL were 9.8% and 14.4%, respectively, and for pooled saliva containing 1.0 µg/dL were 4.9% and 13%, respectively.

Serum cortisol was measured using MP Biomedicals Cortisol Antibody Coated Tube radioimmunoassay kit (MP Biomedicals, Santa Ana, CA). The kit was validated for cattle serum. Serum samples were randomized and thawed at 27 °C. Samples were assayed in duplicate with 25 µL of serum in each tube. The inter- and intra-assay coefficients of variation were 4.5% and 9.3%, respectively.

Statistical Analyses

The MEAN procedure of SAS (Statistical analysis software; SAS Institute Inc., version 9.4, Cary, NC) was used for descriptive statistics with sample size, means, standard deviation, minimum and maximum being reported. The General Linear Model of SAS Statistical Software (SAS) was used to analyze differences of physiological and temperament measures, where the class variable was exit velocity classification and sex. The dependent variables were chute score, rectal temperature, blood lactate (Lactate Pro meter), individual exit velocity, glucose, serum lactate, salivary cortisol, and serum cortisol. Least squares means were computed and statistically separated by the least significant difference method. Means were considered statistically different when the treatment *F*-test was $P < 0.05$. Because there was a significant ($P \leq 0.04$) difference between sexes, Pearson product-moment correlations were calculated by sex. Linear models were analyzed (R-Studio, version 2.15.1) where exit velocity was compared to the variables and combination of variables of plasma lactate, rectal temperature, glucose, salivary cortisol, and serum

cortisol to determine which measure or measures could possibly be used as an objective temperament measure similar to exit velocity. An Akaike information criterion (AIC) was used to analyze the quality of the models (Akaike, 1974). The lowest AIC values are reported for steers and heifers indicating the best candidate linear models to predict exit velocity. Animals were assigned to one of three classifications based on exit velocity. Animals were sorted on exit velocity and the fastest one-third of the animals were classified as fast, the middle-third were medium and the slowest-third were slow. After animals were classified PROC GLM was used to determine if the classifications could be sorted into the same classes as exit velocity by any of the measurements. A discriminate function analysis (SAS 9.4, 2014) was used to determine how often the top candidate model from the AIC classified the animals in the correct exit velocity category. Significance was determined *a priori* at ≤ 0.05 level.

RESULTS AND DISCUSSION

Descriptive Statistics

The purpose of this was to evaluate the relationship between blood lactate measures and physiological biomarkers as a proxy for exit velocity to enable a more objective and practical measure of temperament.

The chute score assigned to animals ranged from 2 to 5 (Table 2). These values are similar to those reported by Boles et al. (2015). It should be noted that there was only one animal, a heifer, that was assigned a chute score of 5. In addition, individual mean exit velocity for steers was 2.21 with a range of 0.31–6.02 m/s and 2.76 for heifers with a range of 0.31–7.12 m/s. The mean exit velocities reported here for steers and heifers were similar to exit velocities reported by Curley et al. (2006) when evaluating Brahman bulls and Burdick, Agado et al. (2011) when evaluating Brahman calves (2.12 m/s \pm 0.05) postweaning. Both of these studies evaluated *Bos indicus* animals, which have been noted to have a more excitable temperament than *Bos taurus* cattle (Burrow and Dillon, 1997; Ferguson et al., 2006; Sant'anna et al., 2012). In mixed breed steers of *Bos taurus* parentage ($n = 1,181$), Vetter et al. (2013) measured exit velocities twice reporting mean exit velocities of 2.9 \pm 0.88 m/s and 3.0 \pm 0.87 m/s, respectively. In addition, Boles et al. (2015) evaluated exit velocities of 154 Angus \times Simmental steer and reported a mean exit velocity of 2.88 \pm 0.77 m/s supporting the data presented here.

Table 2. Descriptive statistics for body weight (WT), chute scores (CS¹), rectal temperature (TEMP), blood lactate as measured by the handheld meter (BLAC²), exit velocity (EV), serum glucose (GLUC³), serum lactate (SLAC), and salivary (SCORT) or serum (BCORT) cortisol for steers and heifers

Steers	<i>n</i>	Mean	SD	Range
WT, kg	82	402.35	84.55	227–581.8
CS ¹	82	2.9	0.56	2.0–4.5
EV, m/s	81	2.21	1.25	0.31–6.02
TEMP, °C	82	39.79	0.45	38.8–41.9
BLAC ² , mM	78	3.47	2.61	BDL ² –13.6
SLAC, mM	82	5.43	4.26	0.16–20.44
SCORT, µg/dL	79	0.18	0.14	0.10–4.9
BCORT, µg/dL	82	1.64	0.94	0.05–0.85
GLUC ³ , mg/dL	82	104.73	33.47	65.6–305.1
Heifers				
WT, kg	109	414.2	79.83	220.0–580.9
CS ¹	109	3.22	0.59	2–5
EV, m/s	108	2.76	1.37	0.31–7.12
TEMP, °C	108	39.93	0.53	38.3–41.5
BLAC ² , mM	108	4.32	2.83	BDL ² –13.0
SLAC, mM	105	6.06	3.79	0.38–19.67
SCORT, µg/dL	107	0.26	0.15	0.05–0.87
BCORT, µg/dL	109	2.13	1.07	0.15–5.03
GLUC ³ , mg/dL	105	112.04	34.16	63.1–295

¹Chute Scores – 1 = Docile, 2 = Restless, 3 = Nervous, 4 = Flighty (Wild) 5 = Aggressive, 6 = Very Aggressive

²Lactate Pro meter lowest detectable limit is 0.07 mM.

³Normal bovine values for glucose 57–79, <https://ahdc.vet.cornell.edu/sects/clinpath/reference/chem.cfm>

Mean measures for blood lactate (Table 2) measured using a Lactate Pro meter were 3.47 mM for steers and 4.32 for heifers (range—below detectable limits 0.07 mM to 13.6 mM) whereas mean serum lactate concentrations were 5.43 mM for steers and 6.06 mM for heifers (0.16 to 20.44 mM). The dissimilarities between the two measures are attributed to differences in sensitivity of measures and techniques used. Two microliters of whole blood is measured in less than 60 s with the Lactate Pro meter whereas serum concentrations were measured with a sensitive ELISA assay. Nonetheless, there is a strong correlation (Tables 4 and 5) between the two measures. This strong correlation between a handheld meter and serum has also been reported by Burfeind and Heuwieser (2012) where measurements from a similar handheld lactate meter were correlated with serum ELISA results with $r = 0.75$ ($P < 0.01$) in lactating cows and $r = 0.98$ in calves ($P < 0.01$).

Mean salivary and serum cortisol concentrations were 0.18 µg/dL for steers and 0.26 µg/dL for heifers (0.05 to 2.13 µg/dL; Table 2). Similar to mean measures for Lactate Pro meter and serum lactate concentrations, mean measures for salivary and serum cortisol were different. Hellhammer et al. (2009) assessed the reliability and relationship of salivary cortisol to the unbound cortisol in blood. They reported differences and concluded

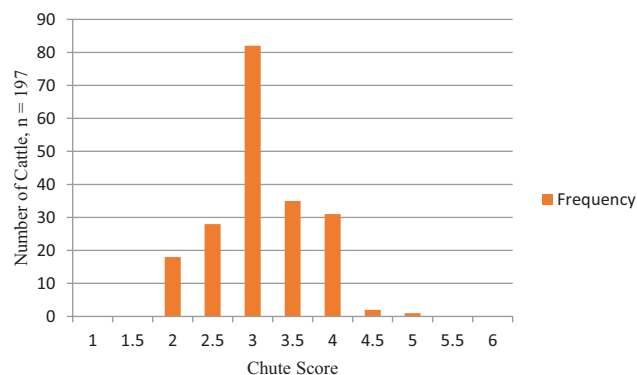


Figure 1. Frequency plot of chute scores for both sexes assigned by a single observer.

that salivary cortisol was distanced from the stress response due to the complexity of factors that modulate the hypothalamic–pituitary–adrenal (HPA) axis, thus reported measures would vary between salivary and serum cortisol concentrations. Nonetheless, Hellhammer et al. (2009) concluded that salivary cortisol was the strongest and most reliable measure for cortisol, when compared to other bodily excretions like milk, urine, and feces ($r = 0.52$, $P < 0.0001$).

Figure 1 shows a frequency plot of chute scores for all animals as assigned by a single observer. The majority of the chute scores were 3 and 3.5, even though exit velocities and physiological responses

suggested greater variation in cattle sampled. Because of the use of the hydraulic squeeze chute, descriptors of the chute scores and subjective observation, there is a question as to the ability of chute score to identify differences that are not extreme. For this research, the feedlot operator was asked to select two pens of “calm” animals and two pens of “excitable” animals as determined by previous handling experience of animals (i.e., entry into feedlot) to achieve extremes in the measurements. As Figure 1 shows, there were no extreme animals, with a tendency for chute scores to be toward the middle of the scale with the mean chute score of 3.1 with an SD of 0.60, even when extremes were anticipated. This tendency to use the center of the scale reduces the variation in the data complicating interpretation and analyses. The chute scores were, therefore, not included in the statistical analyses with the objective measures. These data suggested that chute scores may be more effective at identifying animals that are “violent” but not those that are stressed but not responding overtly. Therefore, the use of the chute scoring system developed in the early 90s has been effective at removing more volatile animals but may not be precise enough to identify stressed animals that may subsequently influence feedlot performance (Grandin, 1993; Ferguson et al., 2006). Other researchers have also questioned chute scores as a temperament measure because of the subjectivity and repeatability between observers (Baszczak et al., 2006; Curley et al., 2006; Benhajali et al., 2010; Boles et al., 2015). Our data suggests that,

within a score, the description is too broad causing animals classed under the same score to have different behavioral (exit velocity) and physiological responses. Using chute scores only allows observers to separate extreme animals whereas temperament differences still exist. With the use of chute scores and temperament scores being implemented by breed associations to calculate expected progeny differences there may be mixed results (Hyde, 2005; American Angus Association, 2010; BIF, 2018). As our chute scores indicate, animals that were classified by the feedlot operator as excitable were not separated by chute scores. Subsequently, if producers were to select heavily for docility or temperament using the chute scoring system, there would be animals that are truly excitable classified as moderate.

Effect of Temperament Classification and Sex on the Physiological Measures

Animals were placed into three different groups based on exit velocity (fast, medium, and slow). The means of individual exit velocity (Table 3) were significantly different between each classification ($P \leq 0.0001$), indicating that the classification was successful in grouping animals that left the chute at different speeds and would suggest temperament classifications were successful. In addition, as exit velocity increased the physiological measure of plasma lactate as measured by the Lactate Pro meter, also increased (Table 3), indicating this

Table 3. Classification of temperament based on exit velocity effect on body weight (WT), chute scores (CS), individual animal exit velocity (EV), rectal temperature (TEMP), blood lactate (BLAC), serum lactate (SLAC), salivary (SCORT) or serum (BCORT) cortisol and serum glucose (GLUC)

Item	Exit velocity class ¹			SEM	P-value
	Fast	Medium	Slow		
<i>n</i> = 197					
WT, kg	452.8 ^a	402.9 ^b	374.7 ^c	10.8	****
CS ²	3.4 ^a	3.1 ^b	2.8 ^c	0.07	****
EV, m/s	4.10 ^a	2.56 ^b	1.06 ^c	0.07	****
TEMP, °C	40.15 ^a	39.78 ^b	39.64 ^b	0.06	****
BLAC ³ , mM	6.4 ^a	3.2 ^b	2.4 ^c	0.3	****
SLAC, mM	9.28 ^a	4.76 ^b	3.86 ^b	0.48	****
SCORT, µg/dL	0.27 ^a	0.21 ^b	0.17 ^b	0.02	****
BCORT, µg/dL	2.25 ^a	1.74 ^b	1.69 ^b	0.14	**
GLUC, mg/dL	129.68 ^a	101.32 ^b	94.63 ^b	4.3	****

Significance = * $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

^{a,b,c}Means within a row that have a different superscript letter differ ($P < 0.05$).

¹Exit velocities were separated by thirds with fastest exit velocities being classified as fast, slowest exit velocities as slow and the middle one-third classed as medium.

²Chute Scores – 1 = Docile, 2 = Restless, 3 = Nervous, 4 = Flighty (Wild), 5 = Aggressive, 6 = Very Aggressive.

³Lactate Pro meter.

biomarker could also group animals in classes similar to exit velocity. Other physiological measures of body temperature, systemic glucose concentrations, serum lactate concentrations, and serum cortisol concentrations were significantly different ($P \leq 0.01$) between fast exit velocity classifications and the other two classes. This indicated that, in the animals classified as fast, there was a significant increase in the physiological response corresponding to faster exit velocities. Finally, salivary cortisol means were significantly higher ($P \leq 0.0001$) in the fast classification than in either the medium or slow classes, also indicating an increased stress response in the animals classed as fast.

Similar classification systems have been reported by [Burdick, Agado et al. \(2011\)](#) where temperament was classed by exit velocities as, calm, intermediate, and temperamental. Brahman calves, classed as calm or intermediate, were more cooperative and less excitable in subsequent handling events. Furthermore, [Boles et al. \(2015\)](#) used exit velocities to class Angus \times Simmental steers as fast, medium, and slow, reporting similar results to [Burdick, Agado et al. \(2011\)](#). Animals within this study had been exposed at least once to the handling facility, suggesting that exit velocities and classification of animals represent the temperament of the animal, not a response to a novel situation. [Lima et al. \(2018\)](#) used serum lactate as well as subjective exit score (1 to 3 score) to assess improved handling methods effect on Nellore steers. They reported that changes in corral arrangement and handling practices significantly affected both cortisol and lactate measurements. They concluded that serum lactate could be used as an evaluation

tool for quality of handling. [Peres et al. \(2014\)](#) related higher serum lactate concentrations to stress in pigs at slaughter. These two research groups suggested that the change in lactate was from physical activity. However, both the data reported here and that reported by [Boles et al. \(2015\)](#) show an increase in blood lactate associated with higher exit velocities supporting the use of chute side measures of lactate as an objective measure of temperament.

Sex had a significant effect on many of the physiological and temperament measures ([Table 4](#)). Weight was not significantly different between steers and heifers, which indicated that the two sexes were at equal stages in the feeding period. However, chute scores, exit velocities, body temperature, blood lactate, and cortisol measures were all significantly different ($P \leq 0.05$) between steers and heifers. The significant difference in chute score ($P \leq 0.001$) and exit velocity ($P \leq 0.01$) between steers and heifers indicate heifers were more excitable than steers. Data for steers were similar to exit velocity data reported by [Behrends et al. \(2009\)](#), [Cooke \(2014\)](#), and [Boles et al. \(2015\)](#). Comparable results for sex differences in exit velocities have been reported by [Hoppe et al. \(2010\)](#), where chute scores and exit velocities were evaluated in Angus, Charolais, Hereford, Limousin, and German Simmental calves. These researchers reported higher chute scores and flight speeds for heifers when compared to steers. [Voisinet et al. \(1997\)](#) also reported heifers to have higher chute scores with more excitable temperaments than steers. In addition, [Gruber et al. \(2010\)](#) reported *Bos taurus* heifers to be more excitable during pen behavior scoring and more reactive to being confined in a chute than

Table 4. Effect of sex on body weight (WT), chute scores (CS¹), exit velocity (EV), rectal temperature (TEMP), blood lactate (BLAC²), serum lactate (SLAC), salivary (SCORT) or serum (BCORT) cortisol and serum glucose (GLUC)

Item	Steers	SEM	Heifers	SEM	P-value
<i>n</i>	87		109		
WT, kg	426.85	9.04	425.33	7.84	n.s.
CS ¹	2.94	2.9	3.24	3.2	***
EV, m/s	2.24	0.14	2.80	0.12	**
TEMP, °C	39.78	0.05	39.93	0.05	*
BLAC ² , mM	3.45	0.31	4.35	0.26	*
SLAC, mM	5.43	0.44	6.05	0.39	n.s.
SCORT, µg/dL	0.18	0.02	0.26	0.01	***
BCORT, µg/dL	1.64	0.11	2.13	0.09	***
GLUC, mg/dL	104.72	3.74	112.04	3.30	n.s.

Significance = * $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

Values are least square means. Significantly different $P < 0.05$.

¹Chute Scores – 1 = Docile, 2 = Restless, 3 = Nervous, 4 = Flighty(Wild), 5= Aggressive, 6 = Very Aggressive

²Lactate Pro meter.

steers. In contrast, other researchers reported no difference in exit velocities between steers and heifers (Burdick et al. 2009; Burdick, Agado et al. 2011; Francisco et al. 2012). Discrepancy in reported data could be due to time during feeding phase the measures were done, the animals in this study were measured in the middle of the feeding phase, which means the handling facility was not a novel experience. Furthermore, due to the commercial setting, dry matter intake was not measured and amount of MGA consumed may have been different and it could contribute to variation in the physiological measurements reported. Researchers have reported that cattle become less agitated as the animals become accustomed to the handling facility (Hall et al., 2011; Lyles and Calvo-Lorenzo, 2014). Moreover, proper cattle management and handling of cattle early and throughout their lifespan is important for reduced stress response to handling (Grandin, 1993; Petherick et al., 2009; Hall et al., 2011). Novel experiences vs. measuring temperament after the cattle have been handled through the system previously contribute to differences in reported studies.

Rectal temperatures were significantly higher in heifers ($P < 0.05$) when compared to steers. Burdick, Carroll et al. (2011) reported the lowest average baseline rectal temperature among calm bulls, slightly above 38.5 °C (SE \pm 0.20) and the highest average baseline rectal temperature occurred in the excitable bulls with rectal temperature near 39.0 °C (SE \pm 0.15). Comparing data to Burdick, Carroll et al. (2011), our average rectal temperature for steers and heifers was higher than bulls classified as excitable. Evaluating rectal temperatures of feedlot cattle can aid in the classification of temperament. Our data along with Burdick, Carroll et al. (2011) support that rectal temperature rises in response to handling. Elevated rectal temperatures reported in this study could be due to handling but also reflects temperament differences. However, this can be complicated if an animal is sick.

Finally, salivary and serum cortisol concentrations were significantly different ($P \leq 0.001$) between steers and heifers, with heifers, having higher circulating salivary and serum cortisol concentrations than steers. Mean cortisol concentrations reported here are similar to mean cortisol concentrations reported by Cooke (2014) for *Bos indicus* steers with what was determined by the researchers to be adequate temperament. Furthermore, means for cortisol concentrations reported by Cooke (2014) were much lower for *Bos indicus* steers than *Bos taurus* heifers. This is similar to data reported

here with steers having lower cortisol response to handling than heifers. However, part of the differences reported by Cooke (2014) could be attributed to the differences in response to handling between *Bos taurus* and *Bos indicus* and differing basal concentrations of serum cortisol (Burrow and Dillon, 1997; Ferguson et al., 2006; Kadel et al., 2006). Stempa et al. (2018) reported similar differences in sheep between intact males and ewes. In addition, the increase in serum cortisol, lactate, and glucose (measure of glycogen depletion) reported by Stempa et al. (2018) led to a reduction in meat quality (higher ultimate pH and darker color) in the carcasses from ewes.

Pearson correlations are reported for both sexes (Tables 5 and 6) because of the significant differences found between heifers and steers. Rectal temperatures were moderately correlated to blood lactate in both heifers and steers; however, the correlation was stronger in steers ($r = 0.497$, $P < 0.0001$) than in heifers ($r = 0.398$, $P < 0.0001$). In addition, rectal temperatures were correlated to metabolites and exit velocity with moderate, highly significant correlation, to glucose, serum lactate, salivary cortisol, and serum lactate. Interestingly, heifers had the highest correlation between rectal temperature and salivary cortisol concentrations ($r = 0.567$, $P < 0.0001$). Gruber et al. (2010) also found a positive correlation between rectal temperature and serum lactate concentrations and between rectal temperature and serum cortisol.

Blood lactate was also significantly correlated to metabolic products and hormone measures. Importantly, blood lactate as measured by the Lactate Pro meter was highly correlated to lactate measured in serum in the laboratory for both sexes. This indicates that the Lactate Pro meter, measuring one drop of whole blood, reflected the lactate measured in the serum at the laboratory bench. This agrees with the validation study of Burfeind and Heuwieser (2012) where they found a strong correlation ($r = 0.98$) between measures from the blood lactate meter and serum lactate measured in a laboratory.

The data presented here indicate that as exit velocity increased both lactate measures increased. These results agree with Coombes et al. (2014) who reported that as flight speed increased, plasma and muscle lactate increased. In addition, Gruber et al. (2010) found animals that reacted adversely to handling and chute restraint had higher plasma lactate at slaughter. Lactate was also moderate to highly correlated ($r = 0.64$) to systemic glucose concentrations. These findings combined with the findings

Table 5. Pearson correlation coefficients among exit velocity (EV), rectal temperature (TEMP), blood lactate (BLAC¹), serum lactate (SLAC), and salivary (SCORT) or serum (BCORT) cortisol and serum glucose (GLUC) for Heifers

	EV	TEMP	BLAC	SLAC	SCORT	BCORT	GLUC
EV	1						
TEMP	0.443 ****	1					
BLAC	0.529 ****	0.398 ****	1				
SLAC	0.534 ****	0.387 ****	0.828 ****	1			
SCORT	0.362 ****	0.568 ****	0.375 ****	0.341 ***	1		
BCORT	0.218 *	0.417 ****	0.330 **	0.468 ****	0.520 ****	1	
GLUC	0.537 ****	0.419 ****	0.644 ****	0.720 ****	0.382 ****	0.272 **	1

Significance = * $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

¹Lactate Pro meter.

Table 6. Pearson correlation coefficients among exit velocity (EV), rectal temperature (TEMP), blood lactate (BLAC¹), serum lactate (SLAC), and salivary (SCORT) or serum (BCORT) cortisol and serum glucose (GLUC) for Steers

	EV	TEMP	BLAC	SLAC	SCORT	BCORT	GLUC
EV	1						
TEMP	0.447 ****	1					
BLAC ¹	0.631 ****	0.498 ****	1				
SLAC	0.591 ****	0.477 ****	0.781 ****	1			
SCORT	0.162	0.445 ****	0.127	0.105	1		
BCORT	0.159	0.445 ****	0.344 **	0.393 ***	0.524 ****	1	
GLUC	0.322 ***	0.540 ****	0.372 ***	0.483 ****	0.214	0.372 ***	1

Significance = * $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

¹Lactate Pro meter

from Gruber et al. (2010) and Coombes et al. (2014) demonstrated that excitable animals mobilized more glucose through glycogenolysis due to increased energy demand in response to stress in the muscle than less excitable counterparts, resulting in elevated lactate and glucose being transported into the blood.

Salivary cortisol was correlated to serum cortisol in both sexes with almost equal r -values ($r = 0.52$). However, the low r -value does not make it a good candidate for replacing serum cortisol as a measure of stress response; therefore, it will not be discussed further. Evaluating the relationship between cortisol to exit velocity and metabolites, exit velocity was not correlated to serum cortisol measures in steers but in heifers there was a positive

correlation between the measures. Curley et al. (2006) evaluated the relationship of temperament measures and cortisol at three different observation times using yearling Brahman bulls and reported a positive correlation between exit velocity and serum cortisol on day zero ($r = 0.26$, $P = 0.04$) and on day 120 ($r = 0.44$, $P < 0.01$). Although in this study, there was no significant correlation between cortisol measures and exit velocity in steers, correlations reported here in heifers were similar to data reported by Curley et al. (2006).

In addition, in heifers, cortisol was moderately correlated to lactate. Serum cortisol was moderately correlated to serum glucose in both steers and heifers. In pigs, Choe and Kim (2014) evaluated the relationship between blood glucose and blood

lactate to serum cortisol levels at exsanguination. They reported moderate correlation coefficients between blood lactate and serum cortisol ($r = 0.55$, $P < 0.0001$) and blood glucose to serum cortisol ($r = 0.43$, $P < 0.0001$). Our data in conjunction with [Choe and Kim \(2014\)](#) and [Curley et al. \(2006\)](#) support that as temperament becomes more excitable the HPA axis is activated, cortisol increases and, therefore, glycogen is converted quickly in the muscle and the lactate produced is shuttled into the blood ([Silbernagl and Despopoulos, 2009](#); [Coombes et al., 2014](#)). Simultaneously, the increased cortisol secretion causes an increased rate of glycogenolysis and therefore a detectable increase in systemic glucose concentrations.

The major objective of this study was to find a simple physiological chute side measure or combination of measures that correlated to an animal's temperament defined by exit velocity and chute score. Chute scores were not included in any of the comparison statistics because there was central tendency for use of the scale resulting in very little variation, therefore, physiological measures were compared to exit velocity. The strongest correlations to exit velocity were rectal temperature, blood lactate measured by Lactate Pro meter, serum glucose, and serum lactate. To improve production efficiency and meat quality, identifying animals with excitable temperaments entering the feedlots would be helpful. For that reason, implementing easy to use, chute side measures would allow producers to objectively categorize their animals for temperament and improvements in ADG and meat quality (tenderness, color) would be achieved ([King et al., 2006](#)). However, to implement these objective measures, models need to be constructed and validated to see if one or a combination of measures can predict exit velocity. If these measures cannot be used as a predictor, how well can they be used as secondary support with exit velocity for determining an animal's temperament?

Modeling for Exit Velocity Using Chute Side Measures

Because of the differences found between chute side measures and exit velocity in steers and heifers, the models were tested separately. Data were randomized and 80% of the data were used for the analysis of the model. Normality of exit velocity was plotted for sexes using histogram and QQplot in R (R-studio, version 0.98.1028); in addition, Shapiro test was used to analyze normality. No transformation was used on exit velocity ([Shapiro and Wilk, 1965](#)) because exit velocity was normally distributed. Generalized additive models were used to evaluate nonlinear relationships, and the models were plotted using gamma plots to visualize response. For both sexes, the chute side measures appeared to have a linear relationship to exit velocity. Salivary cortisol was also included in the analysis because the collection of saliva requires little training and no special equipment for collection. Salivary cortisol appeared to have a near quadratic response to exit velocity with the asymptote near the mean. Because of the nonlinearity of the response and the complex relationship between salivary cortisol and serum cortisol, this model was not analyzed ([Hellhammer et al., 2009](#)).

Linear models were constructed comparing the chute side measures of body temperature, and blood lactate as measured by the Lactate Pro meter to replace exit velocity. An AIC was used to evaluate the strength of candidacy of these chute side measures to predict exit velocity ([Table 7](#)). In steers, the combination of plasma lactate meter measures and rectal temperature had the strongest AIC weight and therefore represents the best fit model. However, in heifers, blood lactate meter and rectal temperature do not have the same strength as in steers. As shown in [Table 7](#), the AIC for just blood lactate meter could also be a candidate and removed strength from the combination of blood

Table 7. AIC values for chute side measures: blood lactate (BLAC) and rectal temperature (TEMP) to predict exit velocity (EV) for steers and heifers

STEERS	AICc	Δ AICc	AICcWt	Cum.WT	LL
BLAC + TEMP	187.30	0.00	0.84	0.84	-89.30
BLAC	190.64	3.34	0.16	1.00	-92.11
TEMP	200.26	12.95	0.00	1.00	-96.93
Null EXIT	212.93	25.63	0.00	1.00	-104.37
HEIFERS					
BLAC + TEMP	243.20	0.00	0.65	0.65	-117.33
BLAC	244.43	1.23	0.35	1.00	-119.06
TEMP	260.37	17.17	0.00	1.00	-127.02
Null EXIT	272.70	29.50	0.00	1.00	-134.27

lactate meter and rectal temperature. However, in heifers, the AIC assumption of delta AIC having a difference of at least two between top and second candidate models was violated. Nonetheless, due to the strength of plasma lactate meter candidacy and its inclusion in the top model the linear model of plasma lactate meter and rectal temperature is still accepted as the top candidate model.

In summary, the AIC concluded the top candidate model to be blood lactate meter measures in combination with rectal temperature to predict exit velocities. The strength of the candidacy of the blood lactate meter along with the ability of the meter to sort animals into three categories similar to exit velocity suggested the plasma lactate meter could be used as an objective measure of temperament in cattle. The discriminate function analysis (Table 8) of the top candidate model of plasma lactate and body temperature was effective at placing animals in fast and slow groups 69.23% and 61.54% of the time, respectively. This means that almost 7 of 10 times the blood lactate and rectal temperature will place the same animals in the fast or excitable category. The question then becomes does blood lactate and body temperature identify animals that have a physiological stress response that do not leave the chute quickly? Will the blood lactate identify animals that are exhibiting a physiological stress response when animals are in the “freeze or hide” mode as opposed to flee? If this is what is occurring then blood lactate measured with a small handheld meter would be a better measure of temperament than exit velocity.

Table 8. Discriminate function analysis for exit classifications¹ using chute side objective measures of blood lactate² and body temperature³

Class	Fast	Medium	Slow	Total
Fast	69.23 %	29.23%	1.54%	100%
<i>n</i>	45	19	1	65
Medium	42.62%	39.34%	18.03%	100
<i>n</i>	26	24	11	61
Slow	10.77%	27.69%	61.54%	100
<i>n</i>	7	18	40	65
Total	40.84%	31.94%	27.23%	100%
<i>n</i>	78	61	52	191
Priors	0.333	0.333	0.333	

¹Exit velocity classifications were derived by sorting exit velocities highest to lowest and splitting into thirds, first third being fast, second third being medium, and last third being slow.

²Blood lactate was measured using a Lactate Pro meter.

³Body temperature was measured using a veterinary digital thermometer fitted with a rectal probe.

CONCLUSIONS

Cattle temperament has a direct impact on efficiency and perception of livestock production in the United States. Previous efforts have laid a foundation of measures in which we can identify excitable animals. However, there is still a need to identify temperaments that do not subjectively fall into an extremely excitable category but still have an impact on handling, feedlot performance, and carcass characteristics. The aim of this study was to identify the relationship of chute side objective measures to physiological markers as well as the correlations of these measures to exit velocity to identify possible objective chute side measures to replace or augment exit velocity as a predictor of an animal temperament. Blood lactate as measured by the Lactate Pro meter was significantly related to exit velocity and when exit velocity was used as a classification, blood lactate concentration was significantly different between the three classes indicating the potential use of blood lactate to sort animals into temperament classes similar to exit velocity. Furthermore, the AIC indicated plasma lactate in conjunction with body temperature was the strongest candidate for predicting exit velocity. The discriminate function analysis indicated plasma lactate and rectal temperature have the potential to become strong objective measures to augment or replace exit velocity to predict an animal's temperament.

Steers and heifers react differently to handling stress as indicated by the significant differences in chute side measures, physiological measures, and exit velocity. The increased excitable behavior expressed in heifers cannot be easily explained by the data presented within this study. Variation in animal's physiological responses to stressors is impacted by differences in homeostatic “set points,” which makes it difficult to model chute side measures capturing a physiological response to exit velocity. Also, changes in timing of measurements, amount of movement or exercise before measurements and the “speed” of muscle lactate clearance can affect how well the small lactate meters can predict exit velocity. Nonetheless, the use of objective chute side measures can greatly improve our selection and removal of temperamental animals. In addition, the use of chute side measures in combination to exit velocity will remove subjectivity from selection criteria.

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LITERATURE CITED

- Akaike, H. 1974. A new look at the statistical model identification. *IEEE*. 19(6):716–723. doi: 10.1109/TAC.1974.1100705
- American Angus Association. 2010. Docility genetic evaluation research By the numbers. American Angus Association, St. Joseph, MO.
- Baird, G. D., J. G. Van Der Walt, and E. N. Bergman. 2007. Whole-body metabolism of glucose and lactate in productive sheep and cows. *Brit. J. Nutr.* 50(02):249. doi: 10.1079/bjn19830094
- Baszczak, J. A., T. Grandin, S. L. Gruber, T. E. Engle, W. J. Platter, S. B. Laudert, A. L. Schroeder, and J. D. Tatum. 2006. Effects of ractopamine supplementation on behavior of British, Continental, and Brahman crossbred steers during routine handling. *J. Anim. Sci.* 84:3410–3414. doi:10.2527/jas.2006-167.
- Behrends, S. M., R. K. Miller, F. M. Rouquette, Jr, R. D. Randel, B. G. Warrington, T. D. Forbes, T. H. Welsh, H. Lippke, J. M. Behrends, G. E. Carstens, et al. 2009. Relationship of temperament, growth, carcass characteristics and tenderness in beef steers. *Meat Sci.* 81:433–438. doi:10.1016/j.meatsci.2008.09.003.
- Benhajali, H., X. Boivin, J. Sapa, P. Pellegrini, P. Boulesteix, P. Lajudie, and F. Phocas. 2010. Assessment of different on-farm measures of beef cattle temperament for use in genetic evaluation. *J. Anim. Sci.* 88:3529–3537. doi:10.2527/jas.2010-3132.
- BIF. 2018. Guidelines for Uniform Beef Improvement Programs. 9th ed. p. 32–33. <https://beefimprovement.org/library-2/bif-guidelines>. Accessed June 2019.
- Boles, J. A., K. S. Kohlbeck, M. C. Meyers, K. A. Perz, K. C. Davis, and J. M. Thomson. 2015. The use of blood lactate concentration as an indicator of temperament and its impact on growth rate and tenderness of steaks from Simmental × Angus steers. *Meat Sci.* 103:68–74. doi:10.1016/j.meatsci.2015.01.003.
- Braga, J. S., Faucitano, L., Macitelli, F., Sant’Anna, A. C., Méthot, S., & da Costa, M. P. 2018. Temperament effects on performance and adaptability of Nellore young bulls to the feedlot environment. *Livest Sci.* 216:88–93. doi 10.1016/j.livsci.2018.07.009
- Burdick, N. C., J. P. Banta, D. A. Neuendorff, J. C. White, R. C. Vann, J. C. Laurenz, T. H. Welsh, Jr, and R. D. Randel. 2009. Interrelationships among growth, endocrine, immune, and temperament variables in neonatal Brahman calves. *J. Anim. Sci.* 87:3202–3210. doi:10.2527/jas.2009-1931.
- Burdick, N. C., B. Agado, J. C. White, K. J. Matheny, D. A. Neuendorff, D. G. Riley, R. C. Vann, T. H. Welsh, Jr, and R. D. Randel. 2011. Technical note: evolution of exit velocity in suckling Brahman calves. *J. Anim. Sci.* 89:233–236. doi:10.2527/jas.2010-2973.
- Burdick, N. C., J. A. Carroll, L. E. Hulbert, J. W. Dailey, M. A. Ballou, R. D. Randel, S. T. Willard, R. C. Vann, and T. H. Welsh, Jr. 2011. Temperament influences endotoxin-induced changes in rectal temperature, sickness behavior, and plasma epinephrine concentrations in bulls. *Innate Immun.* 17:355–364. doi:10.1177/1753425910379144.
- Burfeind, O., and W. Heuwieser. 2012. Validation of handheld meters to measure blood L-lactate concentration in dairy cows and calves. *J. Dairy Sci.* 95:6449–6456. doi:10.3168/jds.2012-5329.
- Burrow, H. M., and R. D. Dillon. 1997. Relationships between temperament and growth in a feedlot and commercial carcass traits of *Bos indicus* crossbreds. *Austr J Exp Agric.* 37(4):407–411. doi: Doi 10.1071/Ea96148
- Cafe, L. M., D. L. Robinson, D. M. Ferguson, B. L. McIntyre, G. H. Geesink, and P. L. Greenwood. 2011. Cattle temperament: persistence of assessments and associations with productivity, efficiency, carcass and meat quality traits. *J. Anim. Sci.* 89:1452–1465. doi:10.2527/jas.2010-3304.
- Choe, J. H., and B. C. Kim. 2014. Association of blood glucose, blood lactate, serum cortisol levels, muscle metabolites, muscle fiber type composition, and pork quality traits. *Meat Sci.* 97:137–142. doi:10.1016/j.meatsci.2014.01.024.
- Coles, L., J. Litt, H. Hatta, and A. Bonen. 2004. Exercise rapidly increases expression of the monocarboxylate transporters MCT1 and MCT4 in rat muscle. *J. Physiol.* 561(Pt 1):253–261. doi:10.1113/jphysiol.2004.073478.
- Cooke, R. F. 2014. Bill E. Kunkle Interdisciplinary Beef Symposium: temperament and acclimation to human handling influence growth, health, and reproductive responses in *Bos taurus* and *Bos indicus* cattle. *J. Anim. Sci.* 92:5325–5333. doi:10.2527/jas.2014-8017.
- Coombes, S. V., G. E. Gardner, D. W. Pethick, and P. McGilchrist. 2014. The impact of beef cattle temperament assessed using flight speed on muscle glycogen, muscle lactate and plasma lactate concentrations at slaughter. *Meat Sci.* 98:815–821. doi:10.1016/j.meatsci.2014.06.029.
- Curley, K. O., Jr, J. C. Paschal, T. H. Welsh, Jr, and R. D. Randel. 2006. Technical note: exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. *J. Anim. Sci.* 84:3100–3103. doi:10.2527/jas.2006-055.
- Ferguson, D. M., D. Johnston, H. M. Burrow, and A. Reverter. 2006. Relationship between temperament, feedlot performance and beef quality. Australian Beef-The Leader Conference:161–165. (Proceedings)
- Finkemeier, M. A., J. Langbein, and B. Puppe. 2018. Personality research in mammalian farm animals: Concepts, measures, and relationship to welfare. *Front. Vet. Sci.* 5. doi:10.3389/fvets.2018.00131.
- Francisco, C. L., R. F. Cooke, R. S. Marques, R. R. Mills, and D. W. Bohnert. 2012. Effects of temperament and acclimation to handling on feedlot performance of *Bos taurus* feeder cattle originated from a rangeland-based cow-calf system. *J. Anim. Sci.* 90:5067–5077. doi:10.2527/jas.2012-5447.
- Grandin, T. 1993. Behavioral agitation during handling of cattle is persistent over time. *Appl Anim Behav Sci.* 36(1):1–9. doi: Doi 10.1016/0168-1591(93)90094-6
- Gruber, S. L., J. D. Tatum, T. E. Engle, P. L. Chapman, K. E. Belk, and G. C. Smith. 2010. Relationships of behavioral and physiological symptoms of preslaughter stress to beef longissimus muscle tenderness. *J. Anim. Sci.* 88:1148–1159. doi:10.2527/jas.2009-2183.
- Hall, N. L., D. S. Buchanan, V. L. Anderson, B. R. Ilse, K. R. Carlin, and E. P. Berg. 2011. Working chute behavior of feedlot cattle can be an indication of cattle temperament and beef carcass composition and quality. *Meat Sci.*

- 89:52–57. doi:10.1016/j.meatsci.2011.03.020.
- Hellhammer, D. H., S. Wüst, and B. M. Kudielka. 2009. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology* 34:163–171. doi:10.1016/j.psyneuen.2008.10.026.
- Holmes, J. H., D. W. Robinson, and C. R. Ashmore. 1972. Blood lactic acid and behaviour in cattle with hereditary muscular hypertrophy. *J. Anim. Sci.* 35:1011–1013. doi:10.2527/jas1972.3551011x.
- Hoppe, S., H. R. Brandt, S. König, G. Erhardt, and M. Gauly. 2010. Temperament traits of beef calves measured under field conditions and their relationships to performance. *J. Anim. Sci.* 88:1982–1989. doi:10.2527/jas.2008-1557.
- Hyde, L. 2005. Limousin Breeders Improve Temperament https://www.beefmagazine.com/mag/beef_limousin_breeders_improve. Accessed August 2019.
- Kadel, M. J., D. J. Johnston, H. M. Burrow, H.-U. Graser, and D. M. Ferguson. 2006. Genetics of flight time and other measures of temperament and their value as selection criteria for improving meat quality traits in tropically adapted breeds of beef cattle. *Austr. J. Agric. Res.* 57(9):1029. doi: 10.1071/ar05082
- King, D. A., C. E. Schuehle Pfeiffer, R. D. Randel, T. H. Welsh, Jr, R. A. Oliphint, B. E. Baird, K. O. Curley, Jr, R. C. Vann, D. S. Hale, and J. W. Savell. 2006. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. *Meat Sci.* 74:546–556. doi:10.1016/j.meatsci.2006.05.004.
- Lima, M. L. P., J. A. Negrão, C. C. P. de Paz, P. H. E. Trindade, and T. Grandin. 2018. Exit speed score and serum lactate can be used as tools to assess improved cattle handling practices. *Livestock Res. Rural Dev.* 30:(8).
- Lyles, J. L., and M. S. Calvo-Lorenzo. 2014. Bill E. Kunkle Interdisciplinary Beef Symposium: practical developments in managing animal welfare in beef cattle: what does the future hold? *J. Anim. Sci.* 92:5334–5344. doi:10.2527/jas.2014-8149.
- Mikesh, L. M., and D. E. Bruns. 2008. Stabilization of glucose in blood specimens: mechanism of delay in fluoride inhibition of glycolysis. *Clin. Chem.* 54:930–932. doi:10.1373/clinchem.2007.102160.
- Petherick, J. C., V. J. Doogan, R. G. Holroyd, P. Olsson, and B. K. Venus. 2009. Quality of handling and holding yard environment, and beef cattle temperament: 1. Relationships with flight speed and fear of humans. *App. Anim. Behav. Sci.* 120(1–2):18–27. doi: 10.1016/j.applanim.2009.05.008
- Sant’anna, A. C., M. J. Paranhos da Costa, F. Baldi, P. M. Rueda, and L. G. Albuquerque. 2012. Genetic associations between flight speed and growth traits in Nellore cattle. *J. Anim. Sci.* 90(10):3427–3432. doi: 10.2527/jas.2011-5044
- Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality. *Biometrika.* 52(3–4):591–611. doi: 0.2307/2333709
- Silberagl, S., and A. Despopoulos. 2009. *Color atlas of physiology.* 6th ed. Thieme, New York (NY).
- Stempa, T., Muchenje, V., Abrahams, A. M., and Bradley, G. 2018. Sex and breed affect plasma glucose, lactate, cortisol, meat quality but not muscle glycolytic potential of Dorper and Merino lambs. *Anim. Prod. Sci.* 58(5):958–964.
- Thomas, C., D. J. Bishop, K. Lambert, J. Mercier, and G. A. Brooks. 2012. Effects of acute and chronic exercise on sarcolemmal MCT1 and MCT4 contents in human skeletal muscles: current status. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 302:R1–14. doi:10.1152/ajpregu.00250.2011
- Turner, S. P., E. A. Navajas, J. J. Hyslop, D. W. Ross, R. I. Richardson, N. Prieto, M. Bell, M. C. Jack, and R. Roehe. 2011. Associations between response to handling and growth and meat quality in frequently handled *Bos taurus* beef cattle. *J. Anim. Sci.* 89(12):4239–4248. doi:10.2527/jas.2010-3790.
- Vetters, M. D., T. E. Engle, J. K. Ahola, and T. Grandin. 2013. Comparison of flight speed and exit score as measurements of temperament in beef cattle. *J. Anim. Sci.* 91:374–381. doi:10.2527/jas.2012-5122
- Voisinet, B. D., T. Grandin, J. D. Tatum, S. F. O’Connor, and J. J. Struthers. 1997. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. *J. Anim. Sci.* 75:892–896. doi:10.2527/1997.754892x