

**INFLUENCE OF TEMPERAMENT ON BOVINE
HYPOTHALAMIC-PITUITARY-ADRENAL FUNCTION**

A Thesis

by

KEVIN OWEN CURLEY, JR.

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2004

Major Subject: Physiology of Reproduction

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ABSTRACT

Influence of Temperament on Bovine Hypothalamic-Pituitary-Adrenal Function.

(December 2004)

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Measures of temperament including exit velocity (EV) and pen score (PEN) and were compared over 3 repeated observations (60-d interval) of yearling Brahman bulls (initial BW = 320 ± 4 kg; n = 66). Exit velocity measures were correlated; EV1 to EV2 ($r = 0.32$, $P = 0.01$), EV1 to EV3 ($r = 0.31$, $P = 0.02$), and EV2 to EV3 ($r = 0.47$, $P < 0.001$). Both EV and PEN were correlated with serum cortisol (CS) within Time 1 and Time 3; EV1 to CS1 ($r = .26$, $P = 0.04$), PEN1 to CS1 ($r = 0.29$, $P = 0.02$), and EV3 to CS3 ($r = 0.44$, $P < 0.001$).

Two-year old Brahman heifer were given an ACTH challenge. The calm (C) and temperamental (T) groups consisted of 6 slow (EV= 1.05 ± 0.05 m/sec) and 6 fast (EV = 3.14 ± 0.22 m/sec) heifers. Prior to ACTH challenge, T heifers had elevated CS (T = 48.97 ± 3.42 , C = 29.60 ± 5.46 ng/mL). Basal CS was higher ($P < 0.001$) in T heifers (18.20 ± 2.63 , C = 4.30 ± 0.58 ng/mL). Following ACTH (0.1 IU ACTH per kg BW) area under the response curve (AUC) was greater ($P = 0.07$) in C heifers (T = 69.08 ± 10.69 , C = 95.87 ± 7.24 ng·h/mL). After declining below basal concentrations, CS in T heifers were again greater ($P = 0.02$) than in C heifers.

The same heifers were subjected to a CRH challenge (0.1 μg bCRH per kg BW). Prior to CRH area under the ACTH curve was greater ($P = 0.025$) in T heifers ($T = 385.72 \pm 49.97$, $C = 239.24 \pm 24.04$ pg·h/mL). Basal ACTH did not differ ($P = 0.10$) between temperament groups. Area under the ACTH response curve was greater ($P = 0.057$) in C heifers ($C = 66.72 \pm 10.65$, $T = 38.11 \pm 6.44$ pg·h/mL).

These data demonstrate that cattle with poor temperament exhibit increased stress responsiveness to handling, increased baseline adrenal function but not increased basal pituitary function, and a muted responsiveness to pharmacological stimulus. Thus functional characteristics of the HPA axis vary with animal temperament.

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INTRODUCTION

Stressor induced activation of the hypothalamic-pituitary-adrenal (HPA) axis results in a cascade of endocrine mediated events that enable coping to the particular stressful stimulus. Hypothalamic release of both corticotrophin-releasing hormone (CRH) and vasopressin (VP) stimulate corticotrophes of the anterior pituitary. Subsequent cleavage of adrenocorticotrophic hormone (ACTH) and release into peripheral circulation enables activation of its primary target tissue, the adrenal cortex. Upon stimulation with ACTH, adrenal cortical tissue releases glucocorticoids (GC) and catecholamines. The primary GC associated with stress responses are cortisol (human and domestic livestock) and corticosterone (rats and mice). As GCs are the endpoint of this HPA response, serum or plasma concentrations of GC can be utilized as physiologic indicators of an ongoing stress response or quantifications of an individual's stress responsiveness.

Fear is a well known stimulus of the HPA axis stress response. Regarding domestic livestock, fear of humans is of concern as many necessary management practices require human-animal interactions. Animal temperament assessments have been utilized to gauge the relative excitability of individual animals to the presence of humans and to specific practices common with animal production schemes. The physiologic mechanisms linked to animal temperament have not been classified. Yet, the adrenal glands have been associated with a fear response since Cannon (1932)

This thesis follows the style and format of the Journal of Animal Science.

described the "fight or flight" reaction to stressors. Increased GC concentrations have been observed in cattle with poor temperament (Fell et al., 1999; Stahringer et al., 1990), however, very little other information exists about the relationship of animal temperament with the HPA axis. Both temperament and stress-induced HPA function have been shown to negatively impact many aspects of beef production. As the cattle industry is one of economic importance to the state of Texas, further investigation into the interactions between cattle temperament and the HPA axis is warranted. With a better understanding of this specific relationship between behavior and physiology, remedies to, or methods of circumnavigating, negative consequences of poor temperament and stress on cattle and the beef industry could be developed.

Thus the objectives of this research include:

1. comparison of temperament assessments, using multiple techniques, over repeated observations;
2. identification of relationships between various temperament appraisals and serum concentrations of cortisol;
3. comparison of adrenal activity following stimulation with exogenous ACTH, in temperamental and calm Brahman heifers;
4. comparison of both pituitary and adrenal activity following stimulation with exogenous CRH, in temperamental and calm Brahman heifers.

LITERATURE REVIEW

Temperament and the Cattle Industry

Primer on Animal Temperament. With the domestication of animals came the observation that individual behavioral responses to man differed greatly from species to species and also within herds or groups. Scott and Fredericson (1951) identified “tameness” and “wildness” as pertained to animal reactions towards man. They defined the term “tameness” as the absence of conflict behavior, and the term “wildness” as the tendency to escape. If nothing else, attention to the specific reactions of animals towards humans enabled handlers to identify those individuals that were easier to work with from those that needed precautions to be taken while handling them. The term “temperament” has also been used to characterize behavior responses to human-animal interactions (Burrow, 1997). However, within the scientific community much misunderstanding has accompanied this term as researchers have used temperament when referring to the nervousness, skittishness, quietness, excitability, individuality, libido, constitution, and emotionality of animals (Stricklin and Kautzscanavy, 1984). If we assume that a fear response underpins animal reactions toward man, then a case could be made for those animals of poor temperament (i.e. individuals with a greater adverse reaction to human-handling) exhibiting a greater fear response in general. Fear responses may arise from social interactions, encounters with novel species and situations, or sudden stimuli that can be visual, auditory, or tactile in nature. Thus, temperament may not only characterize an animal’s response to human handling, but

rather the relative excitability of that individual in general. For the purposes of this discussion “temperament” will refer to the relative ease of eliciting such adverse reactions in an individual animal.

Temperament Assessment. Various methodologies have been devised and implicated in the assessment of cattle temperament for scientific inquiry. As a working chute is commonly used during basic management practices within the cattle industry, many temperament assessment methodologies revolve around cattle behavior while confined to the chute. A scoring technique was developed based on animal reaction to entering the chute or weigh box and subsequent restraint with a head gate (Tulloh, 1961). Using this scoring technique animal temperament was determined to be between 1, docile, and 6, aggressive. An example of a specific description for assigning a particular temperament score with the Tulloh method would be; “3, restless: an animal which moves almost continuously, pulling or pushing on sides of crush; stance is difficult to make observations on; flicks tail frequently, snorts; animal objects to having ear tag handled during identification; may be stubborn.” Iterations of this method, as well as others very similar in nature have been utilized in subsequent research (Dickson et al., 1970; Fordyce et al., 1988; Grandin, 1993). In addition, the heritability of temperament assessed with such methodologies has been shown to be moderately to highly heritable (O’Bleness et al., 1960). Similar scoring methodologies have also been used while the animals were in an enclosed pen rather than confined within the chute (Hammond et al., 1996).

Inherent to all the scoring methods of cattle temperament is the overtly subjective nature of these assessments. Variation between different individuals administering these scores can lead to quite different assessments of cattle temperament. Other non-subjective methodologies have also been utilized to quantify cattle temperament. Some commonly used methods regarded the proximity to an animal that a human could maintain (Purcell et al., 1988). In the flight distance test, the shortest distance a human could come to a stationary animal before it moved away was determined; while, in the approach test the shortest distance to a stationary human that an animal would come was measured. However, these proximity measures were often difficult to obtain, extremely time-consuming to implement under research conditions, and exponentially troublesome when incorporated into routine management practices (Burrow et al., 1988).

Another objective measure of cattle temperament has been described that is relatively easy to implement under both management and research conditions. Burrow et al. (1988) demonstrated that the speed at which cattle exit the working chute was correlated to animal temperament. The cattle that exited the chute with faster velocities were of a more excitable temperament when compared to those that had a slower flight speed and were calmer. The measures of flight speed have been shown to be correlated with measures of flight distance (Burrow, 1997), and have been subsequently utilized to assess cattle temperament in a research setting (Fell, 1999; Petherick, 2002; Petherick, 2003; Burrow, 2004).

Temperament and Beef Production. Relationships between animal temperament and livestock production have been investigated for nearly half a century. Such early

investigations demonstrated the relationships of nervousness to decreased conception rates (Pounden and Firebaugh, 1956) and disproportionately elevated energy requirements (Hafez and Lindsay, 1965). Concerning the cattle industry, an excitable temperament has been shown to negatively impact numerous facets of beef production. Cattle with an excitable temperament exhibited decreased average daily gains when compared with herd mates of a calmer temperament (Petherick et al., 2002; Voisinet et al., 1997b). These differences in gains translated into lower yearling body weights of progeny from sires with an excitable temperament (Burrow and Dillon, 1997). Lower body condition scores have also been associated with poor temperament (Petherick et al., 2003).

Relationships between reduced meat quality and poor temperament have also been evaluated. Tenderness has been shown to be influenced by temperament as Warner-Bratzler shear force measures were greater in steers with an excitable temperament when compared to calmer cattle (Voisinet et al., 1997a). Yield has also been shown to be affected by temperament, as excitable cattle yield less meat due to increased amounts of bruise trim from injuries acquired during transportation (Fordyce et al., 1988). In addition, meat from cattle with an excitable temperament has also been shown to exhibit increased percentages of borderline dark cutters compared to meat from calm cattle (Voisinet et al., 1997a). These studies present a good argument for cattle with excitable temperament being undesirable within the beef industry.

Stress and the Hypothalamic-Pituitary-Adrenal Axis

Primer on Stress. The concept of stress is well recognized in the scientific, medical, and public communities despite its relatively abstract nature and history rife with confusion and controversy. Though first proposed by Hans Selye (1936) and formally investigated for nearly three-quarters of a century, this concept is still best characterized only with a working definition: the biologic response by which an organism is coping to threats to homeostasis, (Moberg, 1999). The quandary that still puzzles stress physiologists today stems from the very foundation of this concept's formulation. An alarm reaction that consisted of at least; an enlargement of the adrenal glands, shrinkage of the thymus, spleen, and lymph nodes, and ulceration to the gastric mucosa, is a non-specific response, (Selye, 1936). Although this response has since been shown to be primarily mediated by the hypothalamus, anterior pituitary, and adrenal glands acting in concert, it can be elicited by a seemingly infinite number of events. The non-specificity of this response has also come under scrutiny (Pacak et al., 1998), since there appear to be finite differences in the specific components of the endocrine stress axis that are activated in response to different stressors.

The term "stressor" is used to qualify any such event that activates the HPA axis, regardless of the magnitude of response and which specific components are stimulated. Unfortunately, the popular connotation of the word "stress" revolves around mental strain, anguish, or anxiety, thus much confusion can accompany explanations of stress biology. While stressors can be of a psycho-neural or psycho-social nature, these are only a subset of possible stressors. A proper discussion of stress should include any and

all factors responsible for the activation of the HPA axis and the physiological consequences thereof.

The Adrenal Glands. Even during Selye's initial work with the alarm reaction, which was later designated (Selye, 1973) the general adaptation syndrome (GAS), the adrenal glands were likely candidates for key involvement in the physiology behind this syndrome. Adrenal enlargement and the loss of both cortical lipoids and chromaffin substance were consistent among activations of the GAS (Selye, 1936). Ablation of the adrenals and subsequent attempts to activate the GAS did not result in typical thymic involution; however, adrenalectomy combined with injections of adrenal extracts did yield characteristic changes in the thymus (Selye, 1956). Thus, adrenal secretions were deemed necessary components of the GAS response. It was demonstrated that multiple steroids could be crystallized from adrenal extracts, but, the preparation most effective in the bioassays utilized to test these purifications was water-soluble (Pfiffner, 1942; Mason, 1964). Confirmation of the steroid nature of adrenal extracts, later deemed corticosteroids, was realized with the partial synthesis of cortisone (Kendall, 1949).

The adrenal glands are composed of steroid producing cortical tissue and catecholamine producing chromaffin tissue, also referred to as the adrenal medulla (Pohorecky and Wurtman, 1971). There is a functional zonation of the adrenal cortex into three compartments; the outermost, mineralocorticoid synthesizing, zona glomerulosa (Kaplan and Bartter, 1962), the glucocorticoid producing zona fasciculata (Stachenko and Giroud, 1959), and the innermost, zona reticularis which synthesizes androgens (Cameron et al., 1969). While both the inner zones of the adrenal cortex,

zona fasciculata and reticularis, have the ability to produce a variety of steroids; the relative degree of production of certain steroids would match their primary functions described above (Griffiths et al., 1963). Concerning the adrenals and GAS, glucocorticoids (GC) are the adrenal cortical steroids that are of primary importance as it is their replacement that maintains adrenalectomized animals in good health (Selye, 1971). During times of basal body maintenance, as well as in response to stressors, GCs have many functions. Such roles may encompass mediation of the immune response (Stenzel-Poore et al., 1993), anti-inflammation mechanisms (Hench et al., 1949), regulation of catecholamine synthesis (Pohorecky and Wurtman, 1971), and intervention of glucose homeostasis (Long et al., 1940). Glucocorticoids have been advocated as physiological indicators of the coping response to stressors since stress-induced activation of the HPA axis ultimately results in increased peripheral GC concentrations. As the early investigation of the stress response was being conducted, the complete picture of the HPA had not been fully realized and so the mechanisms by which the adrenals were regulated in times of stress were still an enigma.

The Role of the Pituitary Gland. The question of adrenal regulation became partially answered when investigation focused on the hypophysis, better known as the pituitary gland. During his early experiments with hypophysectomy, Smith (Smith, 1930) observed significant atrophy of the adrenal cortex, but not the medulla. It was also demonstrated that pituitary extracts stimulated adrenal steroid production and release using an in vitro model (Hechter, 1949). At this time the pituitary extracts that

elicited an adrenal response were commonly referred to as “cortin”, but were later identified as adrenocorticotrophic hormone (ACTH).

In addition to pituitary regulation of the adrenal glands, ideas of a negative feedback system where adrenal hormones inhibited pituitary stimulation of the adrenal glands began to develop. This was first demonstrated when administration of cortical extracts prevented adrenal cortex atrophy in response to large amounts of cortin (Ingle and Kendall, 1937). The actions of ACTH upon the adrenals were shown to occur quickly since the lag time between administration and adrenal cortical secretory activity was merely three minutes (Espiner et al., 1972). The molecular structure of ACTH was first proposed by Bell et al. (1956) but later revised and correctly identified (Riniker et al., 1972) as a single-chain polypeptide consisting of thirty-nine amino acid residues. Relative to the GAS, increased ACTH secretion from the adenohipophysis was observed following stimulation with noxious agents (Sydnor and Sayers, 1954) similar to those used in Selye’s initial work (1936). In the years following these early studies, the actions of ACTH on the adrenal glands during situations of stress have become widely accepted and well understood.

Hypothalamic Control of the Stress Response. Hypothalamic mediation of the anterior pituitary gland was first put forth by Harris (1948) when he suggested that factors from the hypothalamus were transported via a portal blood network, from the median eminence, to elicit actions upon the adenohipophysis. Extracts from the median eminence were demonstrated to increase ACTH secretion rate in median eminence lesioned rats (Royce and Sayers, 1960). The factor that regulates anterior pituitary

release of ACTH, corticotropin-releasing hormone (CRH), has been identified as a peptide and was first sequenced to be a forty-one amino acid residue in ovids (Vale et al., 1981). Stimulation of the pars distalis, by CRH, enhances production of the polypeptide precursor molecule proopiomelanocortin (Childs, 1992) from which ACTH is cleaved (Mains et al., 1977). However, vasopressin (VP), a peptide of hypothalamic origin, secreted from the posterior pituitary, has also been shown to induce ACTH secretion from the adenohypophysis (Martini and Morpurgo, 1955). Both CRH and VP have the ability to regulate functions of the corticotrophes separately but have also been demonstrated to act in synergy to stimulate ACTH release (Liu et al., 1983). Thus, it is the actions of both CRH and VP that constitute the hypothalamic contributions to the HPA axis.

Glucocorticoids, Glucose, and Growth during Stress.

Glucose homeostasis is important during a stress response as additional amounts of energy may be needed by skeletal and cardiac muscle. In addition, alterations in blood glucose concentrations during a stress response may elicit an exaggerated insulin reaction, (Munck et al., 1984). Catecholamines and glucagon constitute the first wave of response by inhibiting insulin-mediated glucose uptake into some tissue types as well as increasing substrates for hepatic gluconeogenesis. Glucocorticoids synergistically assist these response mechanisms during prolonged stress as well as evoking other glucose regulating mechanisms independent of the first response wave (Sapolsky et al., 2000). Insulin is the body's primary hypoglycemic mechanism as it facilitates cellular uptake of glucose through receptor-mediated actions. By ultimately reducing insulin receptor

number, GCs may counteract the actions of insulin; such actions have been demonstrated as dexamethasone decreases insulin receptor substrate-1 in adipose tissue, (Turnbow et al., 1994). Reduction in glucose uptake by adipose, lymphoid and skin tissues, stimulated by GCs may not greatly contribute to increased blood glucose concentrations but may result in catabolism within those tissues (Munck, 1971).

Increased blood glucose concentrations result from GC-aided hepatic gluconeogenesis. The roles of GCs in gluconeogenesis are twofold as they activate enzymes crucial to the gluconeogenic pathway (Pilkis and Granner, 1992), and increase availability of gluconeogenic substrates through lipolysis and proteolysis (Exton, 1987). Assistance of catecholamine-induced triglyceride hydrolysis represents a permissive action of GCs (Lacasa et al., 1988), and can result in increased concentrations of nonesterified fatty acids (Dallman et al., 1993). Concerning protein and gluconeogenesis, GCs have been attributed to cause increased utilization of amino acids for carbohydrate production and subsequently increased blood urea (Long et al., 1940).

As a result of the actions of GCs, growth of a stressed animal is compromised. During times of stress, coping and maintenance become higher priorities than growth and development. The sequestering of glucose into certain cell types limits the energy available to other areas of the body, and ensures the limitation of temporarily unnecessary body processes. Stress reduces body stores of lipids and protein possibly resulting in significant losses over extended periods of distress (Sapolsky et al., 2000). In addition, some physiologic mechanisms of growth are inhibited during periods of stress. Somatomedins are growth factors that play roles in protein synthesis and lipogenesis, as

well as bone growth and are under the control of growth hormone. Circulating concentrations of a common somatomedin, insulin-like growth factor I, decreased as a result of repeated acute stress, (Laugero and Moberg, 2000). The reductions in available energy, necessary building blocks and required physiological mechanisms, which can result from periods of stress, present a biological state not conducive to growth, reproduction, lactation and development.

REPEATABILITY OF MEASURES OF BRAHMAN BULL TEMPERAMENT AND THEIR ASSOCIATION WITH SERUM CORTISOL CONCENTRATIONS

Introduction

Temperament of domestic livestock can be characterized as a fear response to human-animal interactions. Fear responses may also arise from social interactions, encounters with unfamiliar species, or sudden stimuli, which may be visual, auditory, or tactile in nature. Human-animal interactions in cattle production commonly occur through handling coupled with various management practices. In addition, the outdoor housing of cattle provides opportunities for foreign stimuli to impose stress upon the animals. Animals with a calmer temperament will have less of a response to certain stimuli while animals with a wilder temperament will be easily excited and/or exhibit a greater fear response. Animal temperament has been shown to have negative impacts on areas of both dairy and beef production. Cattle with wilder temperaments exhibit lower weight gains (Burrow and Dillon, 1997; Voisinet et al., 1997b), produce tougher meat (Voisinet et al., 1997a), have inhibited milk production (Drugociu et al., 1977; Breuer et al., 2000), and yield increased amounts of bruise trim due to injuries acquired during transportation (Fordyce et al., 1988). Various techniques have been utilized to assess animal temperament; however, most are of a subjective nature and have not been validated for their ability to gauge temperament over the long-term. Our objectives in this study were to compare temperament assessments, using multiple techniques, over

repeated observations, as well as the relationship of the temperament appraisals with serum concentration of cortisol.

Materials and Methods

Animals. Sixty-six yearling, fall-born (2002) American Gray Brahman bulls were utilized to identify the repeatability of temperament measures, assessed by exit velocity, pen score, and chute score, and the relationship of such measures to physiological indicators of stress. The cattle were part of a commercial bull herd owned and managed by J. D. Hudgins Inc., (Hungerford, TX), and remained on the ranch property for the duration of the trial.

Data Collection. On three separate occasions (Time 1, Time 2, and Time 3), with an interval of sixty days between each sampling time, data were collected at a working facility on the J. D. Hudgins ranch. The bulls were transported to the working facility from various locations on the 81 km² property. The cattle were herded through a chute system where they were weighed, assigned a chute score, and timed for exit velocity as they were released from the chute system. The bulls were subsequently herded through a second working chute and restrained with a hydraulic squeeze. While the cattle were restrained, blood samples (15 mL) were obtained via coccygeal venipuncture. Upon exiting the second working chute, the bulls were confined to a pen in small groups where they were assigned pen scores. Blood samples were stored on ice to allow them to coagulate. The blood samples were centrifuged within four hours to harvest serum. Serum was frozen and stored at -20 °C until concentration of CS was determined via RIA.

Temperament Measures. Three methods of temperament assessment were utilized during the data collection. These methodologies included two subjective measures: chute score (CHUTE) and pen score (PEN), and one objective measure of exit velocity (EV). Chute scores (Grandin, 1993) were based on visual appraisal of each bull while it was confined, but not restrained, in a working chute. The scores were based on a 1 to 5 scale, a score of 1 equated to a completely calm animal whereas a score of 5 equated to an extremely excited animal. A more detailed breakdown of the individual scores is provided in Table 1. On all three days of data collection the scores were assigned by the same individual observer to eliminate one potential source of variation. Pen scores (Hammond et al., 1996) were based on visual assessments of each bull while being confined to a pen (5 x 10 m) with a small group of conspecifics (n = 5). The scores were based on a 1 to 5 scale, with a score of 1 equating to a completely calm animal and a score of 5 equating to an extremely excited animal (Table 2). While making the pen score appraisal, the assessor would attempt to approach the bulls to gauge their response. As done for the chute score, pen scores were performed by the same individual throughout the three days of data collection. Exit velocity (Burrow et al., 1988) was determined as the rate at which the animals exited the working chute and traversed a fixed distance (1.83 m). Infrared sensors were used to remotely trigger the start and stop of a timing apparatus, (FarmTek Inc., North Wylie, TX).

Cortisol RIA. Serum concentration of CS were determined on duplicate aliquots of sera samples using a single antibody RIA procedure (see Appendix B-1) that was adapted from Willard et al. (1995b) and utilized: rabbit anti-cortisol antiserum (Pantex,

Table 1. Observations associated with the individual categories of chute scores to evaluate animal temperament (Grandin, 1993).

Chute Score	Description
1	calm – no movement
2	restless shifting
3	squirring, occasional shaking of weigh box
4	continuous vigorous movement and shaking of weigh box
5	4 plus rearing, twisting, or violently struggling

Table 2. Observations associated with the individual categories of pen scores to evaluate animal temperament (Hammond et al., 1996).

Pen Score	Description
1	walks slowly, can be approached slowly, not excited by humans
2	runs along fences, stands in corner if humans stay away
3	runs along fences, head up and will run if humans come closer, stops before hitting gates and fences, avoids humans
4	runs, stays in back of group, head high and very aware of humans, may run into fences and gates
5	excited, runs into fences, runs over anything in its path

Div. of Bio-Analysis Inc., Santa Monica, CA, Cat. #P44) diluted 1:2500; standards made by serial dilution (8000 pg/100 μ L to 3.9 pg/100 μ L) of 4-pregnen-11 β ,17,21-triol-3,20-dione (Steraloids Inc., Newport, RI, Cat. #Q3880-000); and radio-labeled cortisol: 3 H-Hydrocortisone (1,2- 3 H, NEN, Boston, MA, Cat. #NET-185). Unknown cortisol concentrations were calculated using Assay Zap software (Biosoft, Cambridge, UK) and counts per minute (cpm) obtained from a liquid scintillation spectrophotometric beta-counter (Beckman Coulter LS 6500). Cortisol antiserum cross-reactivities were with: corticosterone, 60%; deoxycorticosterone, 48%; progesterone, 0.01%; and estradiol, 0.01%, (determined by Pantex). Interassay and intraassay CV were 9.44% and 9.39%, respectively.

Statistical Analysis. The EV data obtained from the first collection day were transformed into an exit velocity ranking (EV RANK) in order to create a discrete variable based on EV. This ranking was a 1 to 3 scale with 1 representing the bulls slower than one standard deviation from the mean EV and 3 equating to bulls faster than one standard deviation from the mean. Repeated measures ANOVA was conducted using the MIXED model procedure of SAS (SAS Inst., Inc., Cary, NC), for a factorial analysis of time and EV RANK effects on EV and serum concentration of CS. Pearson correlation coefficients were calculated between EV, CS, CHUTE, and PEN, both within and across each of the three points of data collection, using the CORR procedure of SAS.

Table 3. Correlations between bull temperament measures and serum concentration of cortisol, at Time 1. (n = 66)

	Exit Velocity	Pen Score	Chute Score	Cortisol
Exit Velocity	-	r = 0.35 P = 0.005	r = 0.36 P = 0.003	r = 0.26 P = 0.042
Pen Score	-	-	r = 0.512 P < 0.001	r = 0.29 P = 0.019
Chute Score	-	-	-	r = 0.09 P = 0.462

Table 4. Correlations between bull temperament measures at Time 2. (n = 66)

	Exit Velocity	Pen Score	Chute Score
Exit Velocity	-	r = -0.04 P = 0.729	r = 0.20 P = 0.105
Pen Score	-	-	r = 0.40 P < 0.001

Results and Discussion

On the first of three data collection days, all measures of temperament were positively correlated to each other (Table 3). In addition, both PEN and EV were positively correlated with CS, but CHUTE was not. So while the various methodologies for temperament assessment may measure slightly different aspects of animal behavior, they do relate to one another and, in the case of EV and PEN, to increased circulating glucocorticoids. Such relationships did not hold true through subsequent data collections. At Time 2, neither PEN nor CHUTE were related to EV, however, PEN and CHUTE were positively correlated to each other (Table 4). Due to the occurrence of unforeseen but documented external stressors while collecting data concentrations of CS at Time 2 were markedly elevated and thus excluded from our analysis. As a result, comparisons between the various assessments of temperament and physiological stress indicators could not be made for this time point.

Table 5. Correlation between bull temperament measures and serum concentration of cortisol, at Time 3. (n = 66)

	Exit Velocity	Pen Score	Chute Score	Cortisol
Exit Velocity	-	r = 0.10 P = 0.421	r = -0.15 P = 0.233	r = 0.44 P < 0.001
Pen Score	-	-	r = 0.14 P = 0.269	r = 0.25 P = 0.043
Chute Score	-	-	-	r = 0.08 P = 0.511

At Time 3, there were no correlations between any of the temperament assessments (Table 5); however, both PEN and EV were again positively correlated to serum concentration of CS. It is to be noted that the correlation between EV and CS was greater than between PEN and CS, as indicated by both a higher r value and a greater significance level. So while the correlations between different temperament assessment methodologies changed dramatically over the three time points of data collection, the relationship between EV and CS remained constant from Time 1 to Time 3.

Pearson correlation coefficients were calculated for the various temperament parameters across all times of data collection in order to identify the consistency of each method's assessment of temperament. Chute scores were not correlated ($P > 0.3$) to each other at any of the three data collections. Exit velocity at Time 1 (EV1) was positively correlated to both EV2 ($r = 0.32$, $P = 0.011$) and EV3 ($r = 0.31$, $P = 0.015$). In addition, EV2 was correlated ($r = 0.47$, $P < 0.001$) to EV3. Similarly, PEN1 was correlated to both PEN2 ($r = 0.31$, $P = 0.01$) and PEN3 ($r = 0.32$, $P < 0.01$), and PEN2 was correlated ($r = 0.52$, $P < 0.001$) to PEN3. Unlike with the CHUTE, both the measures of EV and PEN were correlated throughout the three points of data collection. Also, it may be of importance that correlations among EV measures, as well as PEN, were strongest between Time 2 and Time 3. One speculation concerning any measure of temperament would be that as the novelty of human-animal interaction decreased so would animal temperament scores, as ascertained through human contact. The greater correlations between the later two measures of temperament may suggest a leveling of each animal's response to human handling, and may in fact be more accurate

assessments of the individual bull's temperament. Concerning serum concentrations of cortisol, CS1 was correlated ($r = 0.62$, $P < 0.001$) with CS3.

We analyzed how the serum concentrations of CS and exit velocities changed over time and relative to the original EV rankings. Over the course of data collections EV was influenced by time ($P < 0.001$) as the mean EV decreased from Time 1 (2.82 ± 0.07 m/sec) to Time 3 (2.11 ± 0.10 m/sec). At Time 2 EV (2.25 ± 0.12 m/sec) differed ($P < 0.001$) from Time 1 but not from Time 3 ($P = 0.25$). The decrease in EV over time supports the idea of animal temperament decreasing with repeated handling; however, the fact that there was no significant change in EV from Time 2 to Time 3 may suggest a limit to such an acclimation to human-animal interactions. Exit velocity was also associated ($P < 0.001$) with the original EV RANK throughout the two subsequent data collections (Figure 1). Thus, the assessments of bulls with a particularly calm temperament (i.e. EV RANK = 1) or rather excitable temperament (i.e. EV RANK = 3) proved to hold true through the next two periods of data collection. Time also influenced ($P < 0.001$) serum concentrations of CS, with a slight decline in mean CS observed between Time 1 (14.56 ± 0.65 ng/mL) and Time 3 (11.12 ± 0.82 ng/mL). Even though these concentrations of CS differ statistically, the biological implications of a 2 to 4 ng/mL difference are difficult to infer. Relative to temperament, concentrations of CS were associated ($P = 0.008$) with EV RANK (Figure 2). The relationship of serum concentrations of CS to measures of temperament remained between Times 1 and 3. As the EV RANK was based on EV from Time 1

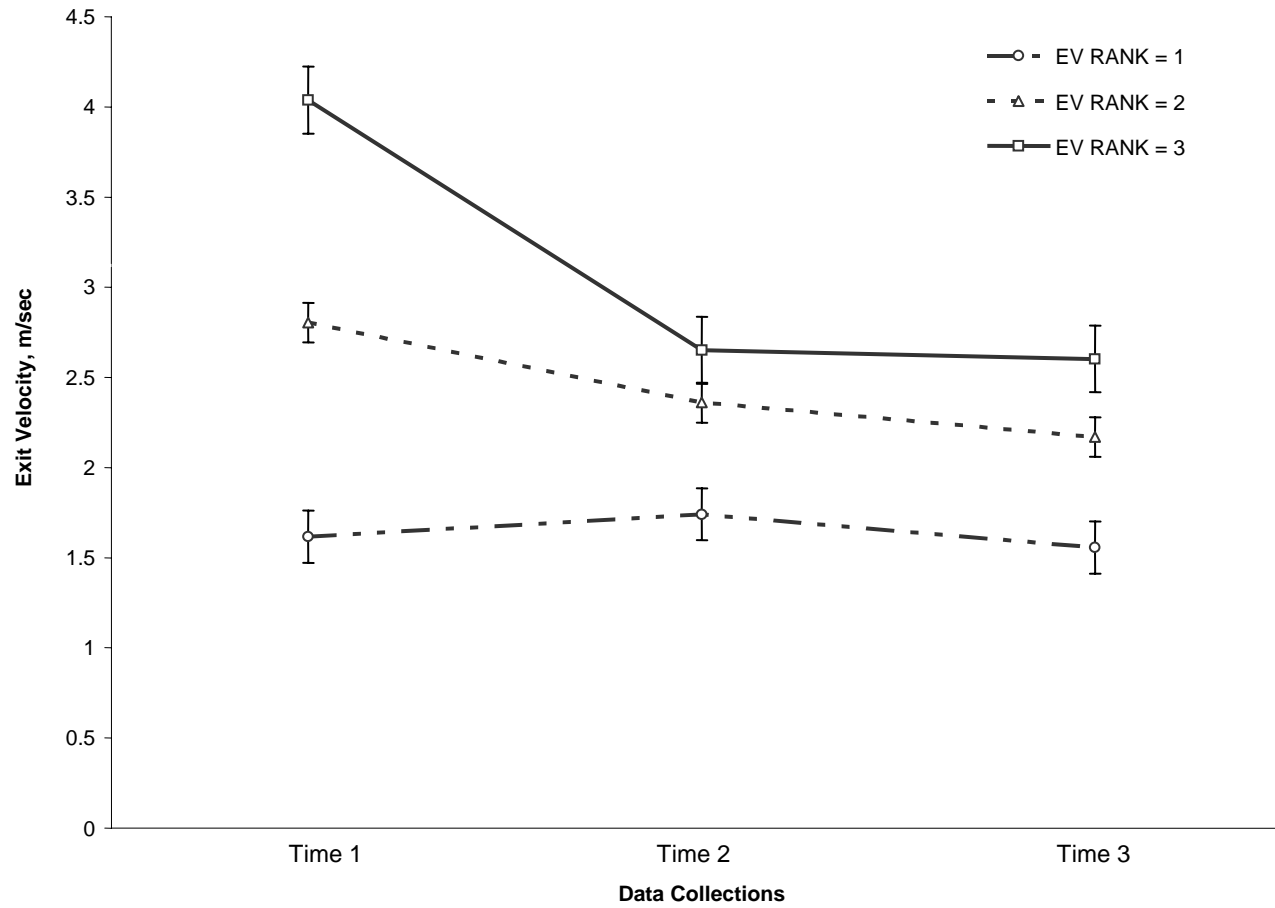


Figure 1. Mean exit velocity measures over the three data collections for each EV RANK.

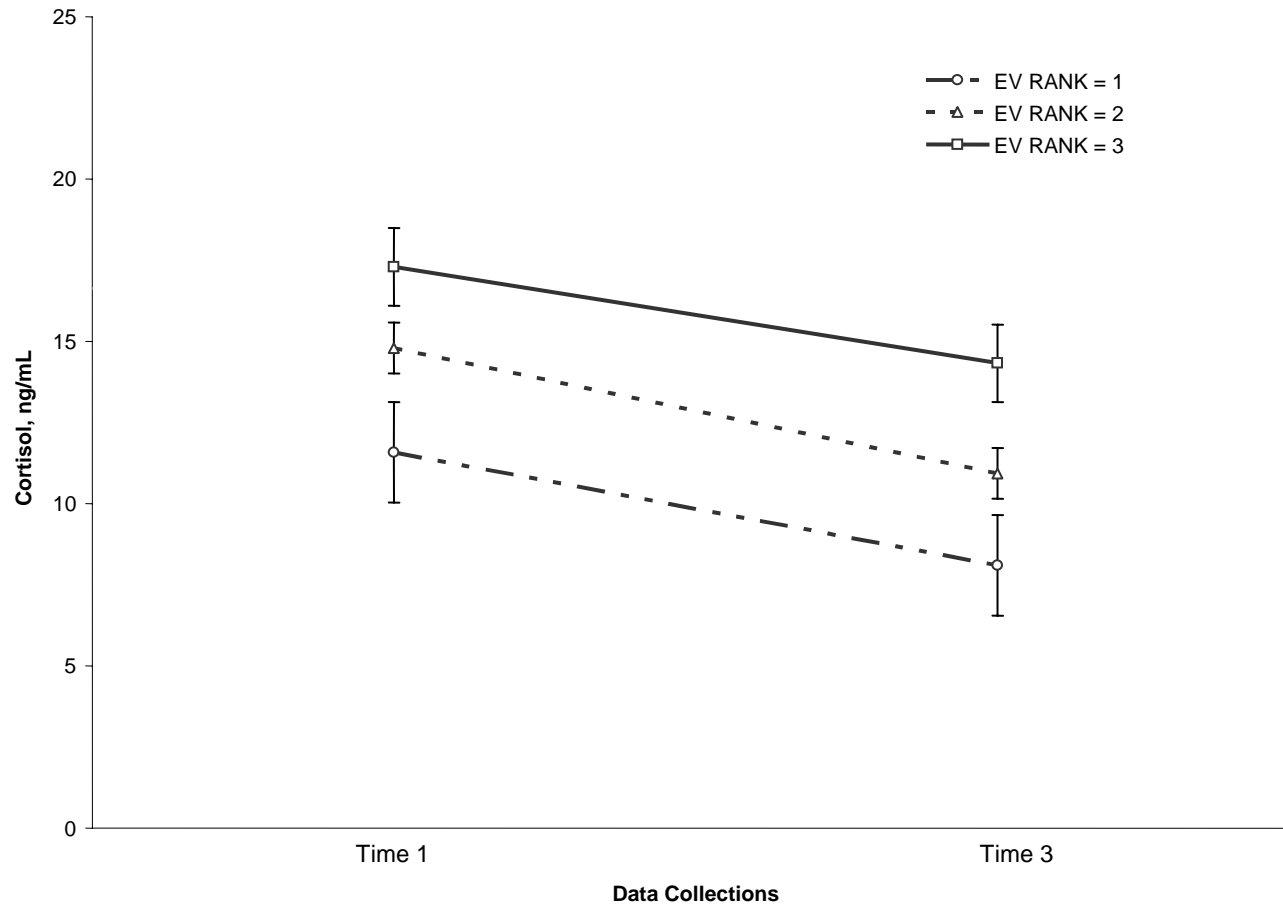


Figure 2. Mean serum concentrations of CS for Time 1 and Time 3 for each EV RANK.

alone, and relative differences in concentrations of CS were observed 120 d later; this measure of temperament appears to be associated with future physiological stress responses.

These data suggest that assessment of cattle temperament with exit velocity measures may be more useful than other subjective methodologies such as pen score or chute score. While all measures of temperament indicated an adaptation of the animals to interactions with humans, both PEN and EV variations were far less affected than CHUTE, over the three points of data collection. As the overt indicators used to qualify temperament with PEN and CHUTE seemed to be less apparent as time progressed, the physiological stress response of the bulls was unchanged. The relationship between EV and serum concentrations of CS was stronger than that of CS and PEN and there was no such relationship between CS and CHUTE. Thus, temperament assessed with such subjective methodologies does not correspond to the stress responses as well as the measure of exit velocity. Exit velocity is therefore a valuable tool for both the assessment of cattle temperament and a possible predictor of temperament through the future of the individual animal's lifetime.

TEMPERAMENT ALTERS BASAL ADRENAL FUNCTION IN BRAHMAN HEIFERS

Introduction

Activation of the hypothalamic-pituitary-adrenal (HPA) axis results in a cascade of endocrine responses that enable coping with stressors. The adrenal steroid hormone cortisol is paramount to the physiological stress response, thus serves as an appropriate biological endpoint in the investigation of HPA function. Cortisol (CS) has been shown to exhibit negative impacts on beef production. Increased CS concentrations can be associated with reduced growth rates (Obst, 1974; Purchas et al., 1980), decreased carcass lean tissue content (Trenkle and Topel, 1978), and increased loss due to dark cutters (Lacourt and Tarrant, 1985).

Similarly, animal temperament has been shown to have negative impacts on aspects of dairy and beef production. Cattle with poor temperament exhibit lower weight gains (Burrow and Dillon, 1997; Voisinet et al., 1997b), produce tougher meat (Voisinet et al., 1997a), exhibit inhibited milk production (Drugociu et al., 1977; Breuer et al., 2000), yield increased amounts of bruise trim due to injuries acquired during transportation (Fordyce et al., 1988), and show signs of a compromised immune system (Fell et al., 1999). Temperament can be characterized as a fear response to novel situations and is commonly associated with human-animal interactions. Fear can stimulate a physiological stress response, thus the link between animal temperament and the HPA is within reason. The observations of elevated CS concentrations in cattle with

poor temperaments when compared to less excitable animals (Fell et al., 1999), further supports such a thesis.

Components of the HPA axis can be pharmacologically activated with the use of exogenous adrenocorticotrophic hormone (ACTH) to mimic pituitary output of ACTH. Subsequently, responses at the adrenal level can be assessed by measuring CS. The use of an ACTH challenge has been shown to be appropriate for investigation into functions of the bovine stress axis (Friend et al., 1977; Zavy et al., 1992; Lay et al., 1996). The objectives in this study were to compare adrenal activity following adrenal stimulation with exogenous ACTH, in temperamental and calm Brahman heifers.

Materials and Methods

Animals. Twelve 2-yr-old, spring-born Brahman heifers (331.12 ± 8.66 kg BW) were utilized to compare HPA activity, following adrenal stimulation. ACTH challenges were conducted at the Texas Agricultural Experiment Station, Overton, over three non-sequential days of 1 wk in May 2003 (mean temperature = 25.9 ± 0.6 °C). Animals were group-housed in a single pen (30 x 10 m) throughout the duration of the trial, except during the 12-h challenge periods. While in the pen, the heifers received free choice access to water and Coastal Bermudagrass hay.

The animals were assigned to one of two treatment groups ($n = 6$) on the basis of temperament. Exit velocity (Burrow et al., 1988) was used to assess animal temperament and was determined by the rate at which the animals exited a squeeze chute and traversed a fixed distance (1.83 m). Infrared sensors we used to remotely trigger the start and stop of the timing apparatus, (FarmTek Inc., North Wylie, TX). The calm (C)

and temperamental (T) treatment groups consisted of the slowest ($EV = 1.05 \pm 0.05$ m/sec) and fastest ($EV = 3.14 \pm 0.22$ m/sec) twenty-fifth percentile of 2-yr-old heifers in the herd ($n = 24$). In addition, pen scores were determined and contributed to assignment of animal to temperament group ($T = 4.33 \pm 0.33$, $C = 1.33 \pm 0.21$).

ACTH Challenges. On challenge days, 4 animals (2 C and 2 T) were confined, but not restrained within segments of a working chute for a period of 12 h. A bolus of 0.1 IU ACTH (Sigma Chemical, St. Louis, MO, Cat. #A 6303) per kg BW, dissolved in physiological saline (0.9%) was administered via jugular cannulas. The temporary indwelling jugular cannulas, inserted 18 h prior to sampling periods, consisted of approximately 15 cm polytetrafluoroethylene tubing (o.d. 1.66 mm; Cole-Palmer, Vernon Hills, IL; Cat. #6417-41 18TW) inserted using 14 gauge thin walled stainless steel biomedical needles (o.d. 2.11 mm) with an additional 15 cm of tubing left outside the animal. The cannulas were maintained in place using branding cement and a patch (12.7 x 5.08 cm) of porous surgical tape. Prior to initiation of blood sampling the cannulas were fit with 2 m extensions of sterile plastic tubing (i.d. 1.59 mm, o.d. 3.18 mm; VWR Scientific, West Chester, PA; Tygon, Cat. #S-50 HL).

Blood samples (15 mL) were collected at 15-min intervals for 360 min prior to and 360 min post ACTH administration (Time 0), with the exception of the final 3 h of sampling when the interval increased to 30 min. Following each sample physiological saline solution (15 mL) and heparinized saline (10 mL) was infused to replace fluid volume and prevent blood clotting in the cannulas, respectively. Samples were immediately placed on an ice bath and centrifuged at 4°C within 3 h of collection.

Serum samples were frozen and stored at -20°C until CS concentrations were analyzed via RIA.

Cortisol RIA. Serum CS concentrations were determined from duplicate samples using a single antibody RIA procedure (see Appendix B-1) that was adapted from Willard et al. (1995b) and utilized: rabbit anti-cortisol antiserum (Pantex, Div. of Bio-Analysis Inc., Santa Monica, CA, Cat. #P44) diluted 1:2500; standards made by serial dilution (8000 pg/100 µL to 3.9 pg/100 µL) of 4-pregnen-11β,17,21-triol-3,20-dione (Steraloids Inc., Newport, RI, Cat. #Q3880-000); and radio-labeled cortisol: ³H-Hydrocortisone (1,2-³H, NEN, Boston, MA, Cat. #NET-185). Unknown cortisol concentrations were calculated using Assay Zap software (Biosoft, Cambridge, UK) and counts per minute (cpm) obtained from a liquid scintillation spectrophotometric beta-counter (Beckman Coulter LS 6500). Cortisol antiserum cross-reactivity: corticosterone, 60%; deoxycorticosterone, 48%; progesterone, 0.01%; and estradiol, 0.01%, (determined by Pantex). Interassay and intraassay CV were 8.06% and 11.6%, respectively.

Statistical Analysis. Repeated measures ANOVA was conducted using the MIXED model procedure of SAS (SAS Inst., Inc., Cary, NC), for a factorial analysis of time and treatment effects on CS concentrations during the PRE (Time -360 through Time 0), RESP (Time 0 through Time 180), and POST (Time 180 through Time 360) periods of the ACTH challenge. A heterogeneous autoregressive covariance structure was used for these analyses. The GLM procedure of SAS was utilized for ANOVA of adrenal function parameters where repeated measures analysis was not necessary. Such parameters included: basal CS concentration (identified as the mean concentration over

the final hour of sampling prior to challenge), peak CS concentrations, amplitude of the CS response to challenge, time to return to basal and area under the curve. The area under the response curve from 0 through 180 min was determined utilizing a method described by Lay et al. (1996):

$$\text{AUC RESP} = \Sigma [(\{(CS_n + CS_{n+1}) / 2\} \cdot h) - (\{(CS_{-15} + CS_0) / 2\} \cdot h)]$$

where h is the time in h between the two CS concentrations. The area of the two samples prior to ACTH challenge was subtracted from the area at each time interval in order to demonstrate adrenal response above basal CS production.

Results and Discussion

There was no day by temperament interaction ($P > 0.1$) for CS concentrations over the 12-h sampling period, thus data were pooled by temperament group for all three challenge days. Hormone profiles for all heifers were similar in that CS concentrations were initially elevated, declined toward basal concentrations, increased following ACTH administration, then preceded to decline again (Figure 3). However, the similarities in glucocorticoid production between the temperament groups, during the PRE period, were restricted to general hormone profiles. The initial response to being handled differed ($P = 0.01$) with temperament as CS concentrations, at Time -360, in T heifers (48.97 ± 3.42 ng/mL) were higher than in C heifers (29.60 ± 5.46 ng/mL). This would suggest that increased adrenal glucocorticoid production, in response to handling, is coupled with the increased behavioral excitability of temperamental cattle. These data concur with other observations of higher plasma CS concentrations found in temperamental cattle when compared to calmer ones (Fell et al., 1999).

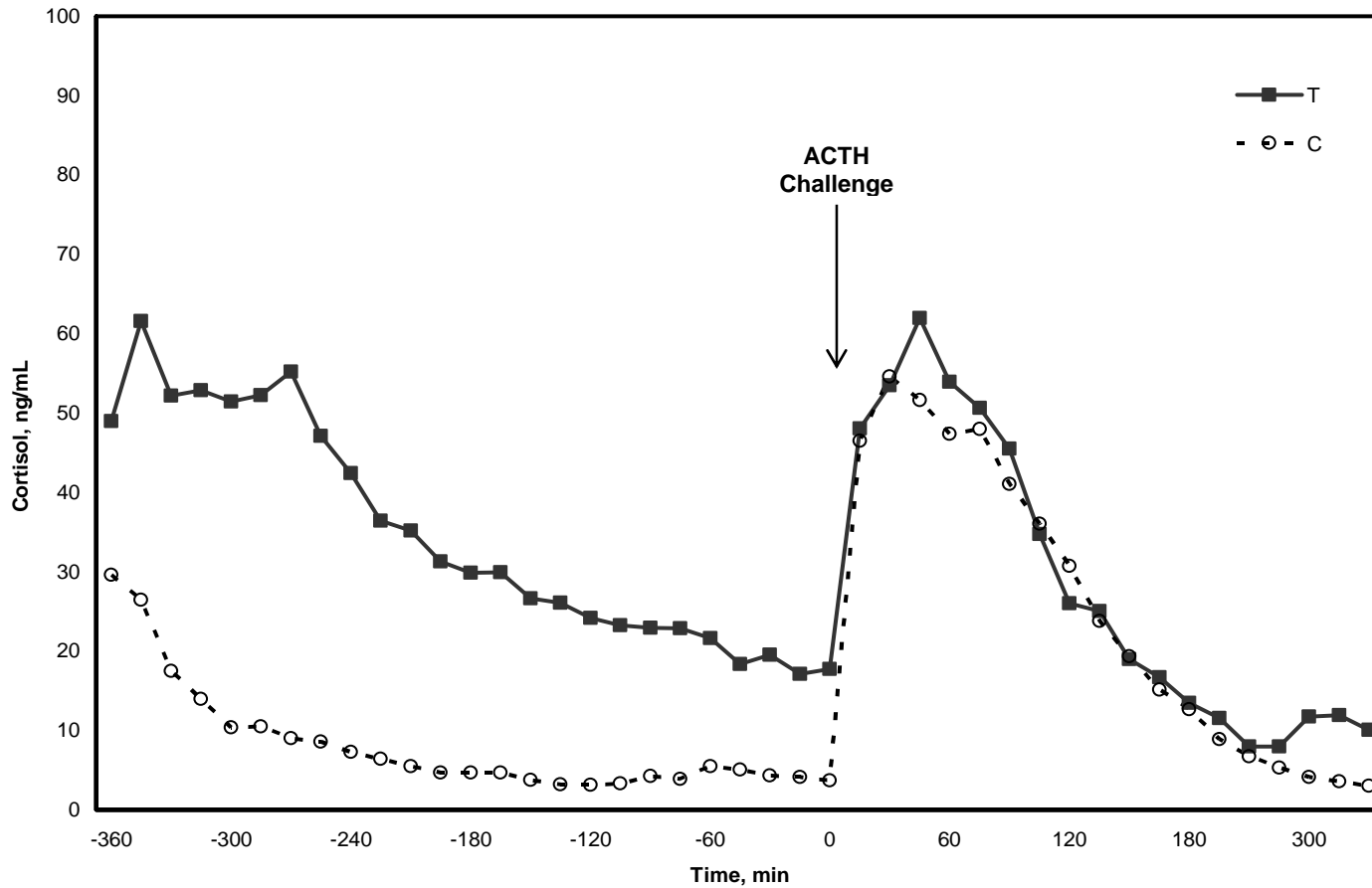


Figure 3. Mean CS concentrations over entire 12-h sampling period for both calm (open circles) and temperamental (solid squares) heifers. Error bars omitted to enhance clarity. ACTH administered at Time 0.

Unlike in the previously mentioned study, repeated sampling allowed for assessing the longevity of this divergence of CS concentrations in cattle with dissimilar temperaments. Focusing on the PRE period there were effects on CS concentrations by time ($P < 0.001$), temperament ($P < 0.001$), as well as a time by temperament interaction ($P = 0.01$). In both calm and temperamental heifers, glucocorticoid concentrations decreased over the six-hour PRE period, signifying an acclimation to both confinement and being in close proximity to human handlers. However, this adaptation seemed slower within the temperamental animals as the decline in mean CS concentrations was retarded in T compared to C heifers (Figure 4). Temperament influenced ($P < 0.001$) basal CS, as concentrations in T heifers (18.20 ± 2.63 ng/mL) were higher than those in C heifers (4.30 ± 0.58 ng/mL). In fact, CS concentrations in T heifers were greater than in C heifers throughout the entire PRE period and only for the initial forty-five minutes were CS concentrations in calmer heifers higher than temperamental animals' basal concentrations (Figure 4). The marked difference in CS concentrations throughout the PRE period indicates increased adrenal responsiveness to both handling and confinement and may suggest an inherent difference in basal HPA function between animals that are calm compared with those that are more temperamental.

Following administration of ACTH, peak CS concentrations did not differ ($P = 0.46$) with temperament and although the amplitude of the CS responses were numerically different ($T = 45.88 \pm 7.65$, $C = 55.14 \pm 1.55$ ng/mL) there was no significant ($P = 0.26$) effect of temperament group on CS amplitude (Figure 5).

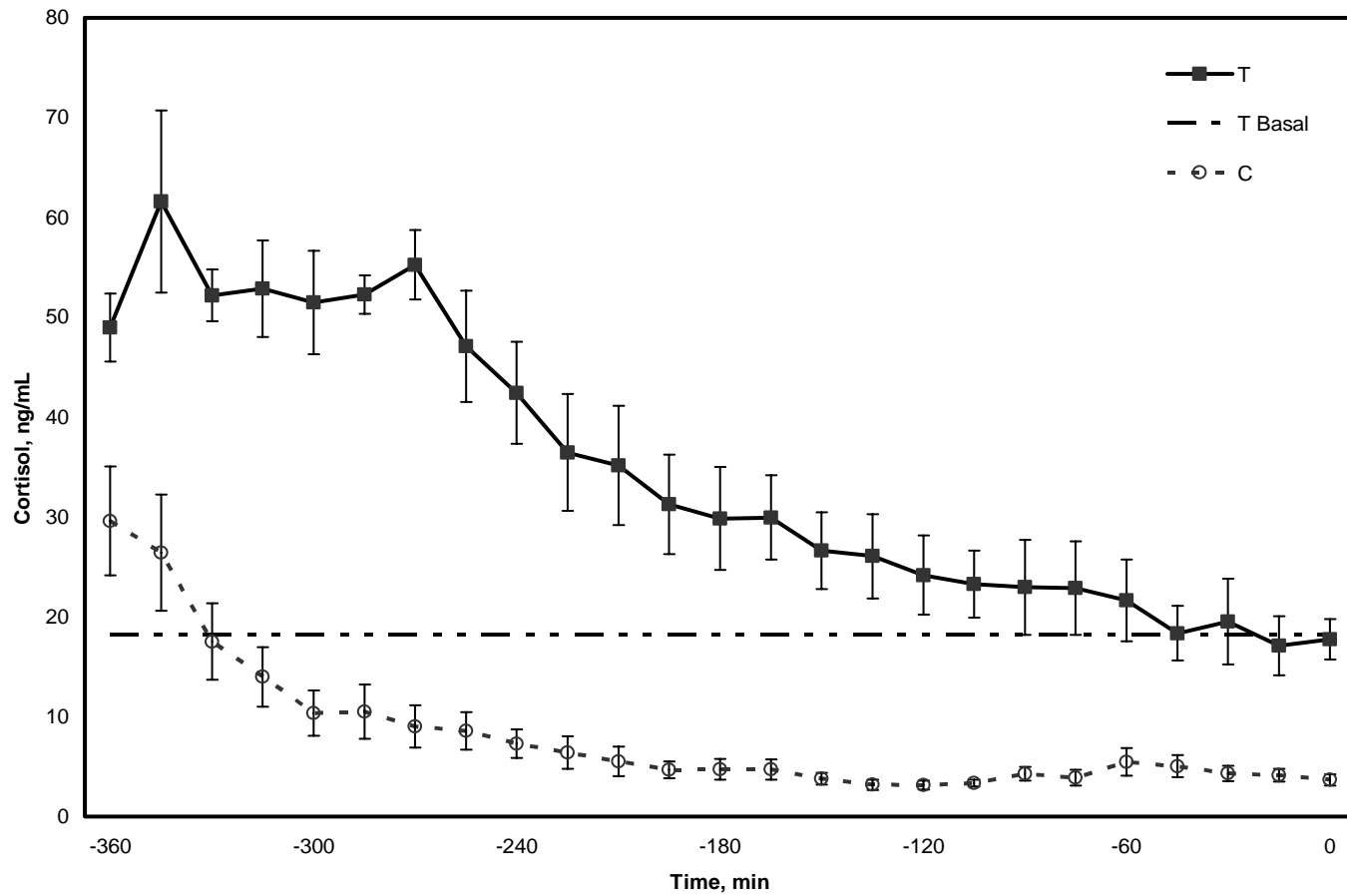


Figure 4. Mean CS concentrations over PRE period for both calm (open circles) and temperamental (solid squares) heifers. Each observation point represents mean \pm SEM. Basal CS for temperamental heifers represented by broken horizontal line.

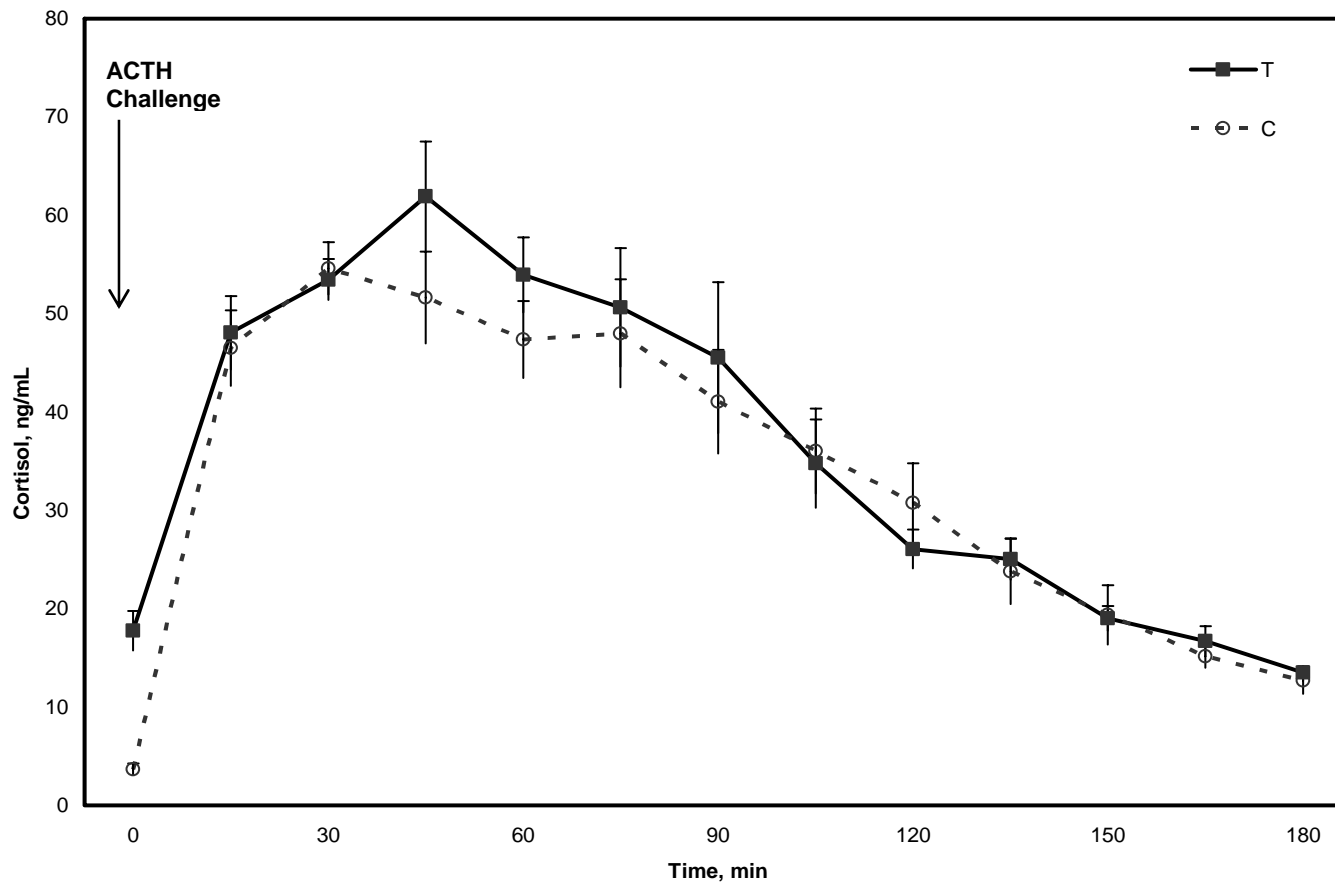


Figure 5. Mean CS concentrations over RESP period for both calm (open circles) and temperamental (solid squares) heifers. Each observation point represents mean \pm SEM. ACTH administered at Time 0.

While the maximum glucocorticoid concentrations did not statistically differ between the treatment groups, there was great divergence in CS production relative to basal concentrations. In response to the challenge there was a 15-fold increase in CS observed in the calm heifers, while only a 4-fold increase in the temperamental ones.

Adrenal output, measured as area under the response curve through the first 180 min post challenge, differed ($P = 0.065$) between temperament groups. As with the amplitude of the glucocorticoid response, the AUC RESP was greater in the calmer heifers ($T = 69.08 \pm 10.69$, $C = 95.87 \pm 7.24$ ng·h/mL). While similar maximal CS concentrations were reached in response to the pharmacological stressor, the overall adrenal response to ACTH above basal concentrations was muted in the temperamental heifers.

Not only was the adrenal response to ACTH muted in the temperamental heifers, but the duration of this response was also limited. Following the adrenal response, the amount of time to return to basal CS concentrations was significantly extended in the calm heifers. The mean time to basal CS was 167.5 ± 17.5 min in the temperamental heifers and 305 ± 12.04 min in the calm group. Granted the higher basal concentrations observed in the temperamental heifers would have taken less time to return to, but if we consider the response as a deviation from basal CS then the calm heifers are clearly in an activated adrenal state for a considerably longer period of time.

During the RESP period CS concentrations were not affected by temperament; however, throughout the final two hours of sampling CS differed ($P = 0.019$) between the calm and temperamental heifers (Figure 6). It was during this POST period that the

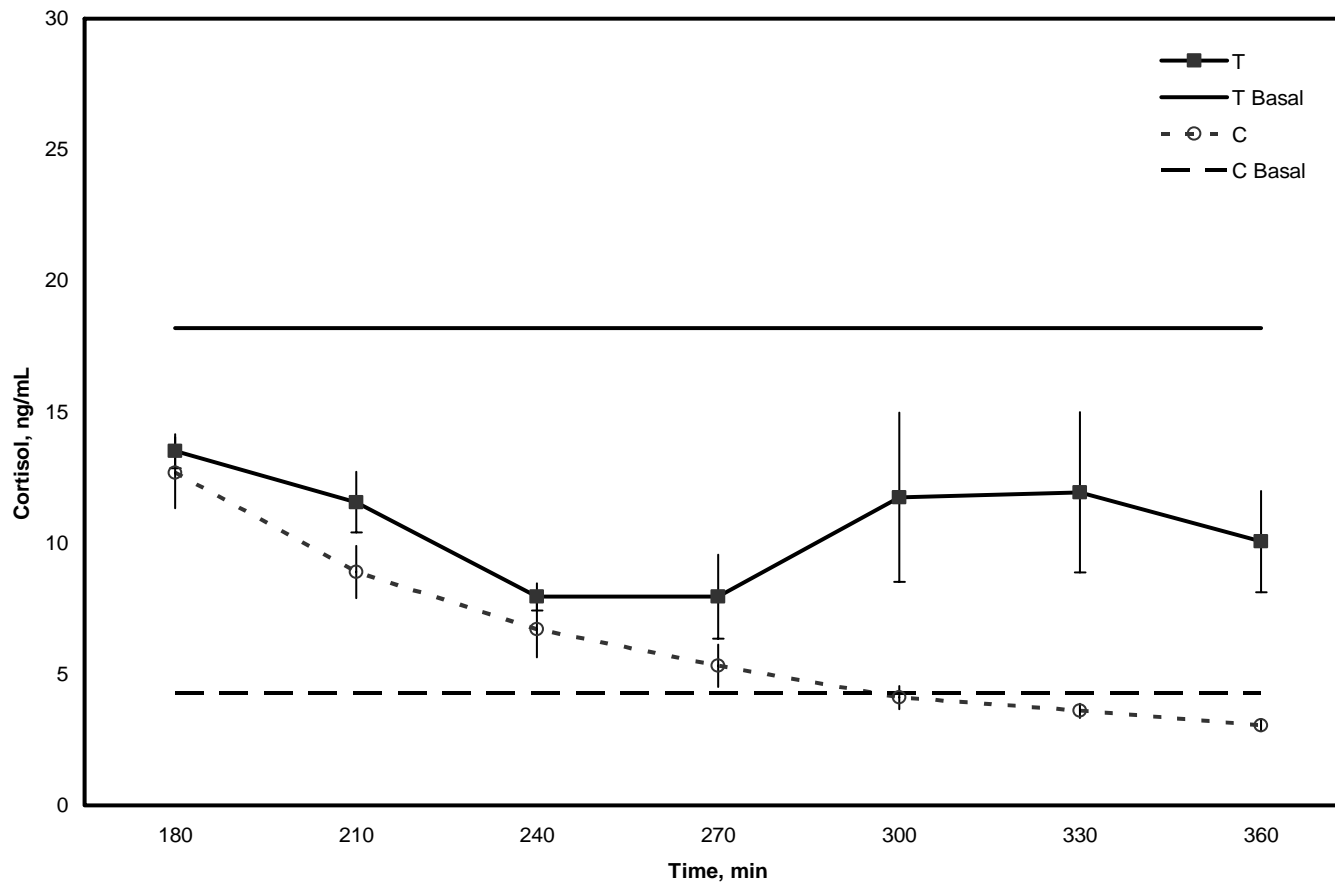


Figure 6. Mean CS concentrations over PRE period for both calm (open circles) and temperamental (solid squares) heifers. Each observation point represents mean \pm SEM. Basal CS for temperamental and calm heifers represented by solid and broken horizontal lines respectively.

CS concentrations dipped below basal in the temperamental heifers and returned to basal in the calm group. At 270 min post ACTH challenge the CS concentrations in the temperamental heifers were entering a refractory period and beginning to elevate. This, coupled with a continued decline of CS in the calm heifers, led to a divergence in circulating glucocorticoids between temperament groups with mean CS in T heifers (11.25 ± 2.20 ng/mL) higher than in C heifers (3.59 ± 0.28 ng/mL) during the final hour of sampling. The increase in CS observed in the temperamental heifers may be indicative of a return to basal concentrations following the overshoot caused by the post challenge decline.

These data suggest a link between cattle temperament and adrenal function. The response to handling was greater in the temperamental heifers compared to the calm heifers, as indicated by comparisons of initial serum concentrations of CS. In addition baseline adrenal CS output was larger in the temperamental cattle. Following adrenal stimulation with exogenous ACTH a subdued response was observed in the temperamental heifers as shown by comparisons of AUC and time to return to basal concentrations of CS. However these analyses of these may reflect the higher basal CS concentrations rather than actual adrenal responsiveness to ACTH challenge. For example, stimulation with ACTH would cause concentrations of CS to reach a peak then decline towards baseline, yet as this baseline was greater in the temperamental heifers this obviously would have taken less time to accomplish. The observation of increased basal concentrations of CS, in the temperamental cattle, is most intriguing as it may suggest a state of chronic stress associated with animal temperament.

TEMPERAMENT OF BRAHMAN HEIFERS AFFECTS PITUITARY AND ADRENAL RESPONSES TO CORTICOTROPIN-RELEASING HORMONE

Introduction

Traditionally, temperament of domestic livestock is characterized as a fear response to human-animal interactions. Animal temperament negatively impacts both dairy and beef production. Cattle with poor temperament exhibit lower weight gains (Burrow and Dillon, 1997; Voisinet et al., 1997b), produce tougher meat (Voisinet et al., 1997a) possess inhibited milk production (Drugociu et al., 1977; Breuer et al., 2000), yield increased amounts of bruise trim due to injuries acquired during transportation (Fordyce et al., 1988), and show signs of a compromised immune system (Fell et al., 1999) when compared with herd mates.

The adrenal steroid hormone cortisol (CS) has been shown to exhibit similar negative impacts on beef production. Increased serum concentrations of CS can be associated with reduced growth rates (Obst, 1974; Purchas et al., 1980), decreased carcass lean tissue content (Trenkle and Topel, 1978), and increased loss due to dark cutters (Lacourt and Tarrant, 1985). It seems intuitive that temperament and stress responsiveness be linked as fear responses and activation of the hypothalamic-pituitary-adrenal (HPA) axis are not mutually exclusive. In addition, elevated exogenous concentrations of CS have been detected in cattle with poor temperament when compared to less excitable animals (Fell et al., 1999).

The HPA axis can be pharmacologically activated with the use of exogenous corticotrophin-releasing hormone (CRH) to mimic hypothalamic CRH output. Subsequently, responses at both the pituitary and adrenal levels can be assessed by measuring adrenocorticotrophic hormone (ACTH) and CS, respectively. The use of a CRH challenge has been shown to be appropriate for investigation into functions of the bovine HPA axis (Veissier et al., 1999; Fisher et al., 2002; Gupta et al., 2004). Our objectives in this study were to compare pituitary and adrenal activity following stimulation with exogenous CRH, in temperamental and calm Brahman heifers.

Materials and Methods

Animals. Twelve 2-yr-old, spring-born Brahman heifers (331.12 ± 8.66 kg BW) were utilized to compare HPA axis activity, following pituitary stimulation with CRH, in temperamental and calm cattle. CRH challenges were conducted at the Texas Agricultural Experiment Station, Overton, over three non-sequential days of 1 wk in April (mean temperature = 18.17 ± 1.22 °C). Animals were group-housed in a single pen (30 m x 10 m) throughout the duration of the trial, except during the 12-h challenge periods. While in the pen, the heifers received free choice access to water and Coastal Bermudagrass hay.

The animals were divided into 2 treatment groups ($n = 6$) on the basis of temperament. Exit velocity (Burrow et al., 1988) was used to assess animal temperament and was determined by the rate at which the animals exited a squeeze chute and traversed a fixed distance (1.83 m). Infrared sensors were used to remotely trigger the start and stop of the timing apparatus, (FarmTek Inc., North Wylie, TX). The calm

(C) and temperamental (T) treatment groups consisted of the slowest ($EV = 1.05 \pm 0.05$ m/sec) and fastest ($EV = 3.14 \pm 0.22$ m/sec) twenty-fifth percentile of 2-yr-old heifers in the herd ($n = 24$). In addition, pen scores were determined and contributed to assignment of animal to temperament group ($T = 4.33 \pm 0.33$, $C = 1.33 \pm 0.21$).

CRH Challenges. On challenge days, 4 animals (2 C and 2 T) were confined, but not restrained within segments of a working chute for a period of 12 h. A bolus of 0.1 μ g bCRH (Peninsula Laboratories, San Carlos, CA, Cat. #8568) per kg BW, delivered in physiological saline (0.9%), was administered via jugular cannula. The temporary indwelling jugular cannulas, inserted 18 h prior to sampling periods, consisted of approximately 15 cm PTFE tubing (o.d. 1.66 mm; Cole-Palmer, Vernon Hills, IL; Cat. #6417-41 18TW) inserted using 14-gauge thin walled stainless steel biomedical needles (o.d. 2.11 mm) with an additional 15 cm of tubing left outside the animal. The cannulas were maintained in place using branding cement and a patch (12.7 x 5.08 cm) of porous surgical tape. Prior to initiation of blood sampling the cannulas were fit with 2-m extensions of sterile plastic tubing (i.d. 1.59 mm, o.d. 3.18 mm; VWR Scientific, West Chester, PA; Tygon, Cat. #S-50 HL) to facilitate longer range sampling and minimize disturbance of heifers as blood samples were collected.

Blood sample (10 mL) collection intervals were 15 min throughout the 12-h period except for the initial 30 min and final 180 min of the post-challenge period; where the sampling intervals were 5 min and 30 min, respectively. Sample coagulation was prevented by 17.6 mg of EDTA solution. Following collection of each sample, physiological saline solution (15 mL) followed by heparinized saline (10 mL) was

infused to replace fluid volume and prevent blood clotting in the cannulas, respectively. Samples were immediately placed on an ice bath and centrifuged at 4°C within 3 h of collection. Plasma samples were frozen and stored at -20°C until ACTH and CS concentrations were analyzed via specific RIAs.

Corticotropin RIA. Plasma concentration of ACTH was determined from duplicate samples using a double antibody RIA procedure (see Appendix B-2) that was adapted from Willard et al. (1995a). All samples were determined with a single assay. This assay utilized: 1:2000 dilution of IgG-ACTH-1 rabbit anti-(1-24)ACTH (IgG Corporation, Nashville, TN) as a primary antibody; goat anti-rabbit gamma-globulin (Calbiochem, La Jolla, CA, Cat. #539845) diluted 1:20, as the secondary antibody; standards made through serial dilutions (100 pg/100 µL to 0.05 pg/100 µL) of h,r(1-24)ACTH (Peninsula Laboratories, San Carlos, CA, Cat. #8741); and radio-labeled ACTH: ¹²⁵I h(1-24)ACTH (ICN Biomedical, Carson, CA, Cat. #07106125). Unknown ACTH concentrations were calculated using Assay Zap software (Biosoft, Cambridge, UK) and intraassay CV was 8.30%.

Cortisol RIA. Plasma concentrations of CS were determined from duplicate samples using a single antibody RIA procedure (see Appendix B-1) that was adapted from Willard et al. (1995b) and utilized: rabbit anti-cortisol antiserum (Pantex, Div. of Bio-Analysis Inc., Santa Monica, CA, Cat. #P44) diluted 1:2500; standards made by serial dilution (8000 pg/100 µL to 3.9 pg/100 µL) of 4-pregnen-11β,17,21-triol-3,20-dione (Steraloids Inc., Newport, RI, Cat. #Q3880-000); and radio-labeled cortisol: ³H-Hydrocortisone (1,2-³H, NEN, Boston, MA, Cat. #NET-185). Unknown cortisol

concentrations were calculated using Assay Zap software (Biosoft, Cambridge, UK) and cpm obtained from a liquid scintillation spectrophotometric beta-counter (Beckman Coulter LS 6500). Cortisol antiserum cross-relativities: corticosterone, 60%; deoxycorticosterone, 48%; progesterone, 0.01%; and estradiol, 0.01%, (determined by Pantex). Interassay and intraassay CV were 8.98% and 8.25%, respectively.

Statistical Analysis. Repeated measures ANOVA was conducted using the MIXED model procedure of SAS (SAS Inst., Inc., Cary, NC), for a factorial analysis of time and temperament effects on ACTH and CS concentrations during the PRE (Time - 360 through Time 0), RESP 1 (Time 0 through Time 30), RESP 2 (Time 30 through Time 180), and POST (Time 180 through Time 360) periods of the CRH challenge. A heterogeneous autoregressive covariance structure was used for these analyses. The GLM procedure of SAS was utilized for ANOVA of adrenal function parameters where repeated measures analysis was not necessary. Such parameters included: basal ACTH and CS concentrations (identified as the mean concentration over the final hour of sampling prior to challenge), peak hormone concentrations, amplitude of the ACTH and CS response to challenge, time to return to basal and area under the curve.

The area under the response curve from 0 through 180 min was determined utilizing a method described by Lay et al. (1996):

$$\text{AUC RESP} = \sum [(\{(CS_n + CS_{n+1}) / 2\} \cdot h) - (\{(CS_{-15} + CS_0) / 2\} \cdot h)]$$

where h is the time in h between the two ACTH or CS concentrations. The area of the two samples prior to CRH challenge was subtracted from the area at each time interval in order to demonstrate pituitary and adrenal responses above basal hormone production.

Area under the curve prior to CRH stimulation was also calculated for both ACTH and CS concentrations. As there was no response above basal concentrations to measure, the area was simply calculated as follows:

$$\text{AUC PRE} = \Sigma \left(\left\{ \frac{\text{CS}_n + \text{CS}_{n+1}}{2} \right\} \cdot h \right)$$

Results and Discussion

Data were pooled from the three challenge days as there was no day by temperament interaction ($P > 0.1$) for either ACTH or CS concentrations. Both corticotropin and cortisol were initially elevated, then declined over the first six hours of sampling in all animals. Hormone concentrations rapidly increased following the administration of CRH then again decreased to a state comparable to pre-challenge observations and continued as such throughout the remainder of blood sampling (Figures 7 and 8).

Pre-challenge Period. Comparison of mean ACTH concentrations, in the first blood sample obtained, revealed only numerical differences ($P = 0.22$), with ACTH in T heifers (163.71 ± 28.59 pg/mL) being higher than in the calmer ones (124.31 ± 8.74 pg/mL). Similarly, CS concentrations at this time point did not statistically differ ($P = 0.15$), yet they too were numerically greater in the temperamental animals (T = 85.96 ± 10.46 , C = 60.19 ± 12.82 ng/mL). Significant variation in hormone concentrations within the temperamental cattle contributed to the lack of statistical difference. One explanation for this relatively large variation in initial ACTH concentrations could stem from the differences in elapsed time from when the individual heifers were first handled

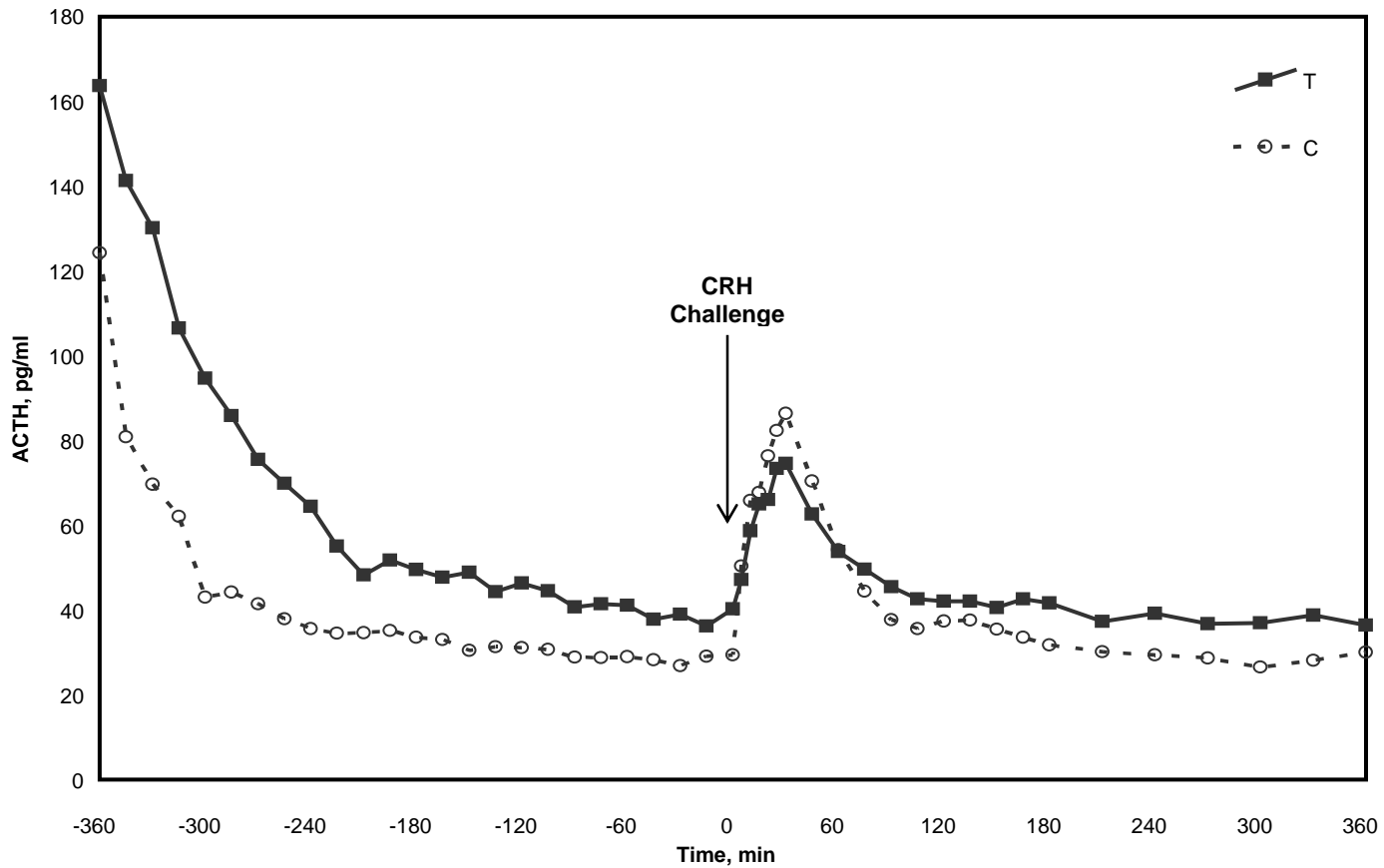


Figure 7. Mean ACTH concentrations over entire 12-h sampling period for both calm (open circles) and temperamental (solid squares) heifers. Error bars omitted for clarity. CRH administered at Time 0.

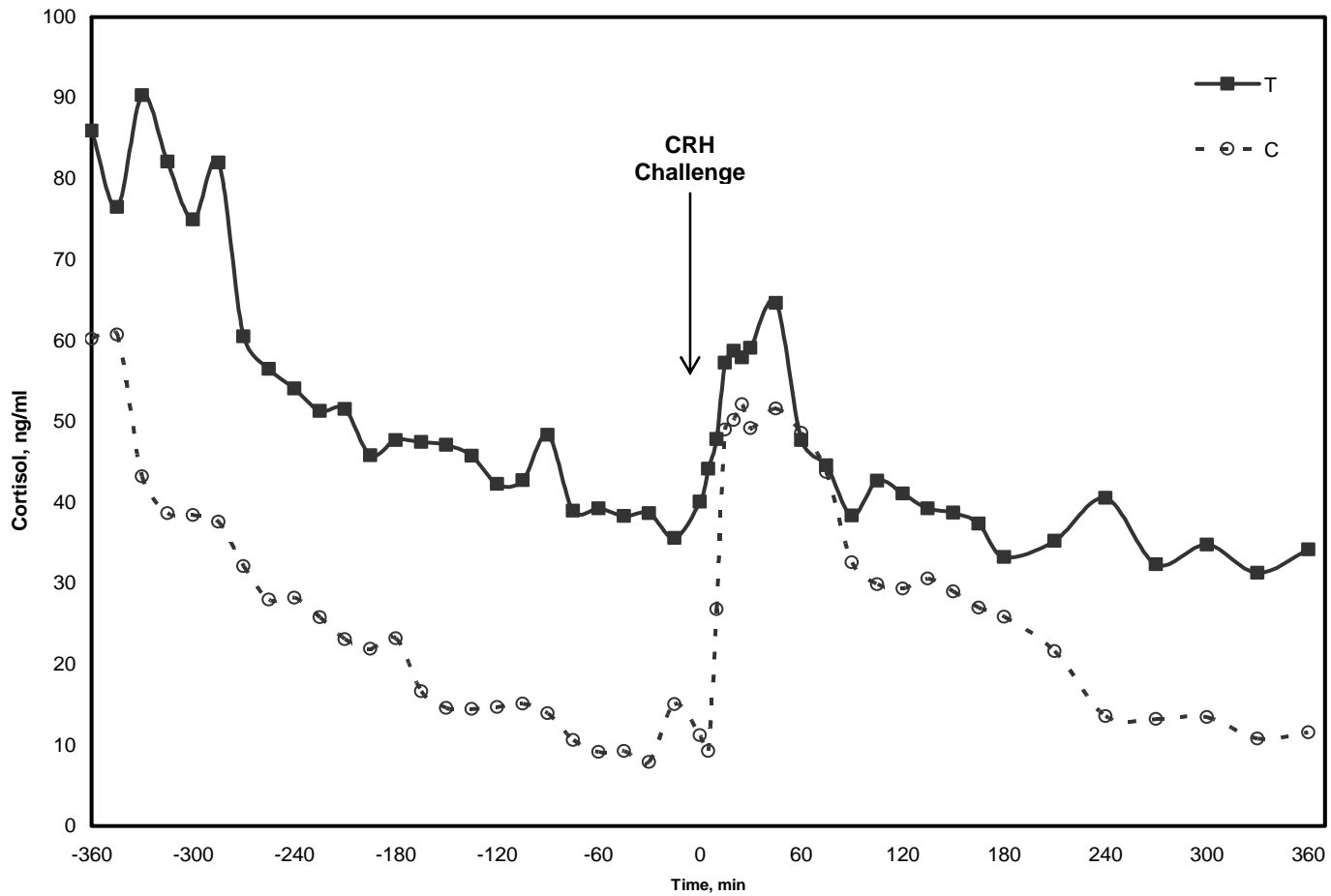


Figure 8. Mean cortisol concentrations over entire 12-h sampling period for both calm (open circles) and temperamental (solid squares) heifers. Error bars omitted to enhance clarity. CRH administered at Time 0.

until the first blood sample was collected. While the stressor (i.e. guiding the animals into the chute system) occurred at the same time, the lag between stressor and blood sampling may have differed by approximately 20 min. Pituitary response has been observed in as few as 2 min post-stressor, (Sydnor and Sayers, 1954). Therefore, temporal synchronization relative to the experimental challenge may have limited our ability to analyze stress responses to the initial handling, by comparison of hormone concentrations at single time points, between the temperament groups, particularly for ACTH.

Analysis of ACTH concentrations over the entire PRE period (Figure 9) revealed effects of both time ($P = 0.002$) and temperament ($P = 0.012$) but not a time by temperament interaction ($P = 0.15$). Cortisol concentrations over these 6 h (Figure 10) were also influenced ($P < 0.001$) by time and temperament, and in addition, exhibited a trend ($P = 0.088$) for a time by temperament interaction. Cortisol concentrations in the temperamental animals remained in an elevated state for a longer duration following the initial stressors when compared to the calmer heifers. Cortisol was declining within the first 30 min of sampling in the C heifers, yet concentrations in the temperamental animals did not begin to decrease until the 90th min of sample collection. This prolonged duration of elevated cortisol concentrations in the T heifers may explain the time by temperament interaction observed; however, it is important to note that a similar retardation in the decline in ACTH concentrations was not observed.

Measures of area under the curve also demonstrated effects of animal temperament on stress hormone concentrations throughout the pre-challenge period. For

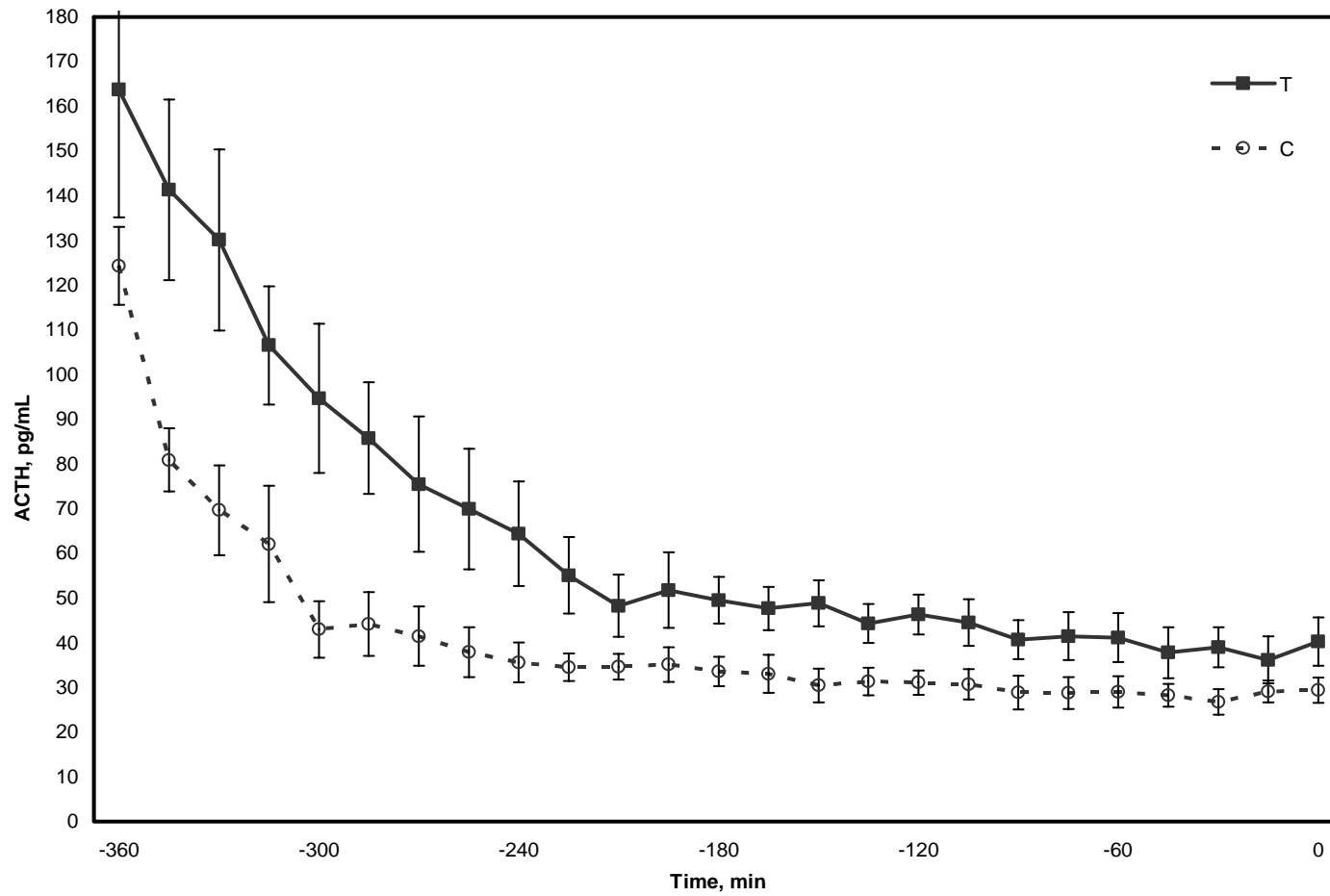


Figure 9. Mean ACTH concentrations over PRE period for both calm (open circles) and temperamental (solid squares) heifers. Each observation point represents mean \pm SEM.

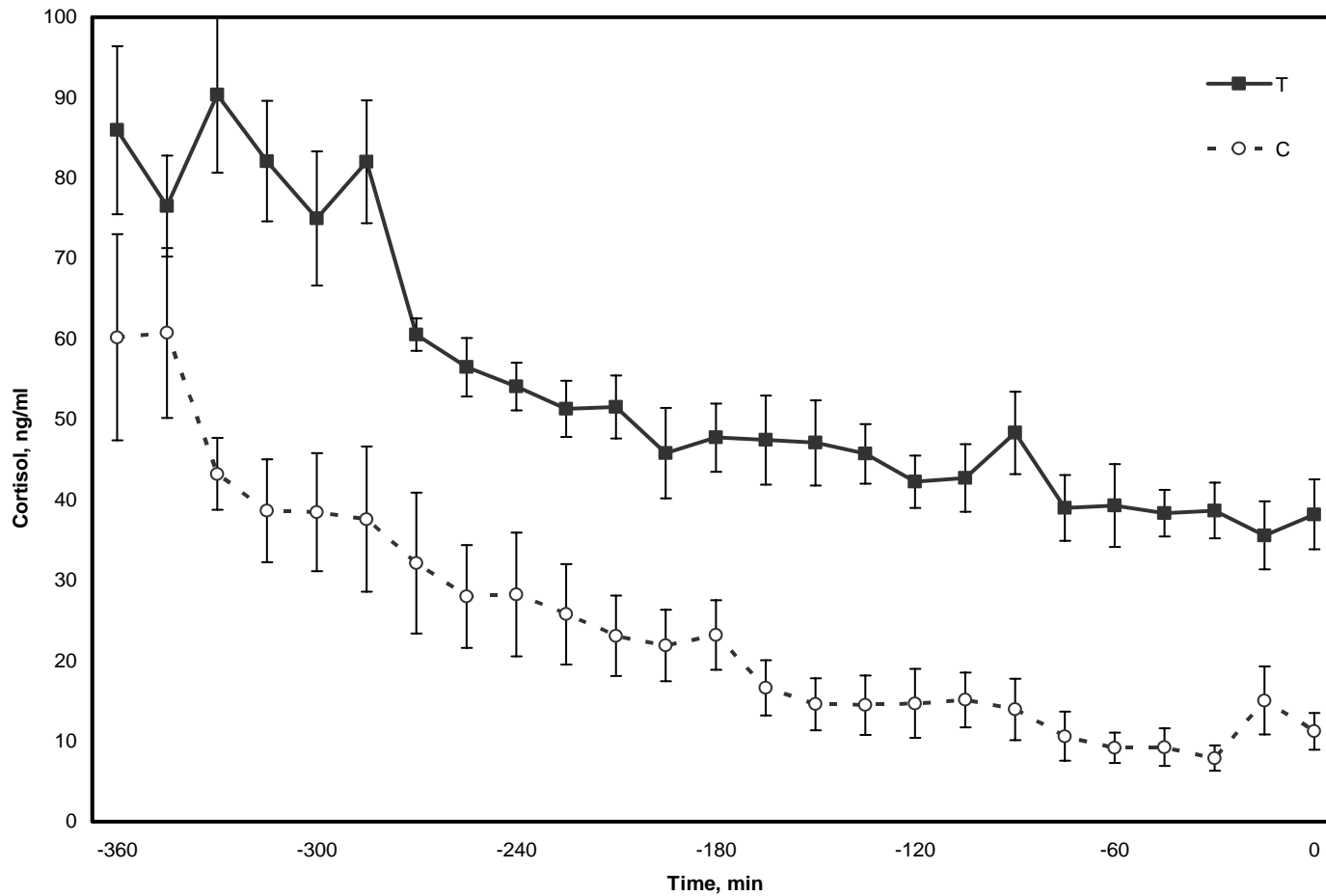


Figure 10. Mean cortisol concentrations over PRE period for both calm (open circles) and temperamental (solid squares) heifers. Each observation point represents mean \pm SEM.

ACTH concentrations, temperament influenced ($P = 0.025$) area under the curve with the area being greater for the temperamental cattle ($T = 385.72 \pm 49.97$, $C = 239.24 \pm 24.04$ pg·h/mL). Also, the area under the CS curves for the temperamental heifers was larger ($P < 0.001$) than that of the calmer animals ($T = 324.94 \pm 11.51$, $C = 144.52 \pm 23.93$ ng·h/mL). While pituitary function over the entire 6-h PRE period was elevated in the temperamental heifers, basal ACTH was not influenced ($P = 0.104$) by temperament as concentrations were only numerically higher in the temperamental cattle ($T = 38.86 \pm 5.12$, $C = 28.51 \pm 2.69$ pg/mL). However, basal CS differed ($P < 0.001$) with animal temperament as concentrations in the T heifers (38.01 ± 3.73 ng/mL) were much higher than in the C group (10.52 ± 2.25 ng/mL). While there was no statistical difference in basal pituitary output in calm and temperamental heifers, there may be biological relevance to such differences at the adrenal level. Corticotropin receptors have been shown to be extremely sensitive to low dose ACTH concentrations as in vitro work has demonstrated increased steroidogenesis in cultured bovine adrenal cells in response to ~ 25 pg/mL ACTH, (Nishikawa et al., 1996).

Post-challenge Period. Repeated measures analysis of ACTH concentrations over the 6-hour post-challenge period (Figure 11) showed no difference ($P > 0.1$) between temperament groups. In response to CRH, peak ACTH concentrations were numerically higher in the calm heifers ($C = 90.09 \pm 10.08$, $T = 71.34 \pm 10.51$ pg/mL) but these data were not statistically different ($P = 0.23$). However, the amplitude of the pituitary output following CRH challenge differed ($P = 0.047$) with animal temperament. The change from basal ACTH concentrations was far greater in the calmer heifers

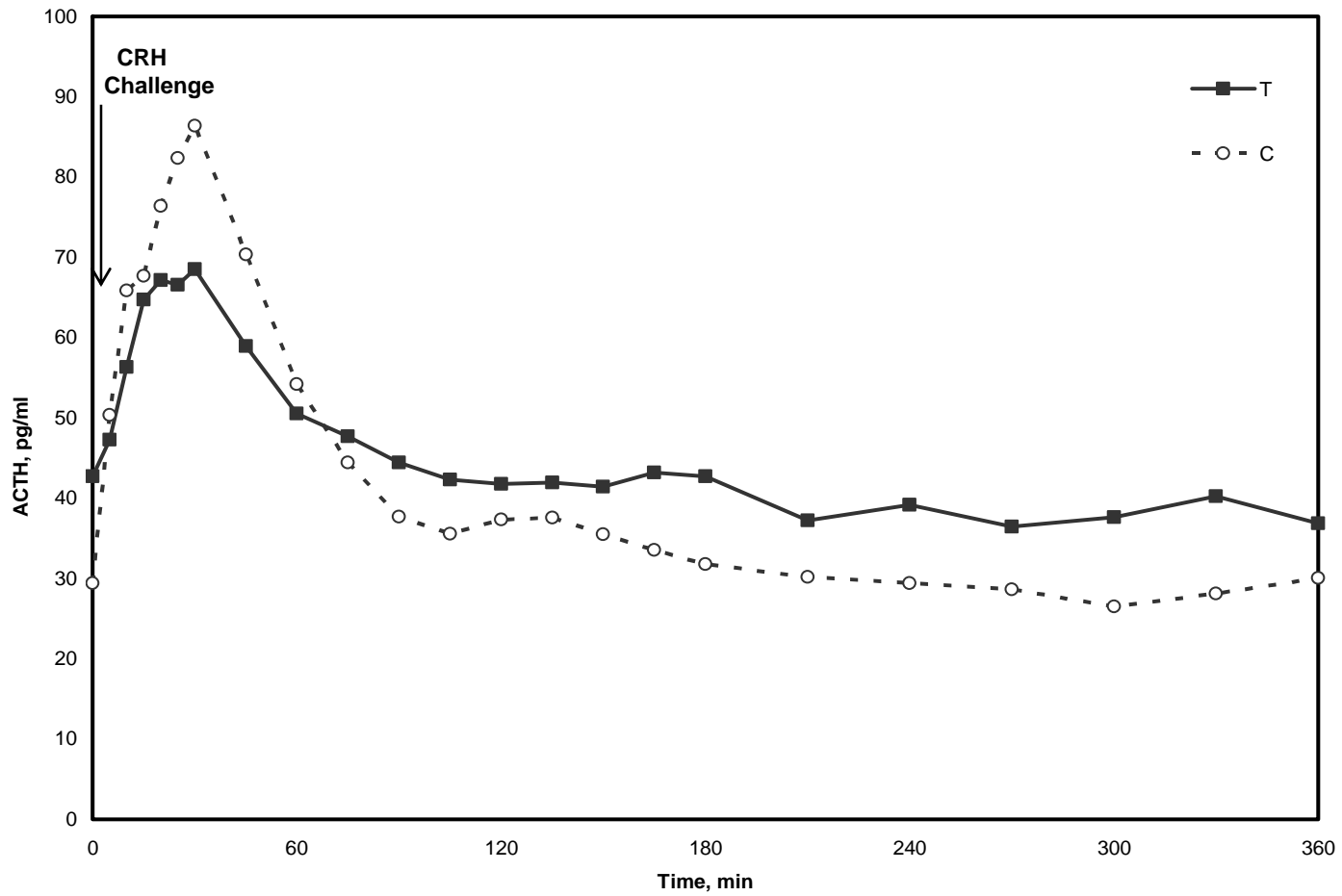


Figure 11. Mean ACTH concentrations over RESP period for both calm (open circles) and temperamental (solid squares) heifers. Error bars omitted to enhance clarity. CRH administered at Time 0.

(61.54 ± 11.05 pg/mL) than observed in the temperamental animals (29.69 ± 7.18 pg/mL). Pituitary response characterized by area under the ACTH curve also differed ($P = 0.057$) with temperament. Concurrent with the amplitude of the corticotropin response, the ACTH areas under the response curve were larger in the calm cattle ($C = 66.72 \pm 10.65$, $T = 38.11 \pm 6.44$ pg·h/mL). The suppressed pituitary response in the temperamental heifers was most likely a result of feedback inhibition by glucocorticoids. The T heifers demonstrated greater adrenal activity in terms of magnitude and duration following the stress of initial handling, as well as elevated baseline CS output. Either of which have been shown to reduce pituitary response to stress stimuli.

Cortisol concentrations during the 6-h post challenge period (Figure 12) were influenced ($P < 0.05$) by time as well as temperament. In addition, time by temperament interactions ($P < 0.05$) were observed over the first 30 min and final 3 h of the post-challenge sampling period. Within the first 30 min, this interaction can be attributed to the relatively sharp increase in CS concentrations observed in the calm heifers following CRH administration. During the final three hours, concentrations in the temperamental heifers had already declined to basal and remained static while continuing to regress in the calm animals, resulting in a significant two-way interaction.

Peak CS concentrations did not differ ($P = 0.22$) between the two temperament groups. Although similar to the pituitary response the amplitude of the adrenalcortical output was greater ($P = 0.01$) in the calm group than in the T heifers ($C = 51.47 \pm 4.53$, $T = 30.96 \pm 4.27$ ng/mL). Also, AUC comparisons revealed a larger ($P = 0.01$) adrenal response in the C heifers ($C = 77.23 \pm 7.59$, $T = 34.89 \pm 11.15$ ng·h/mL).

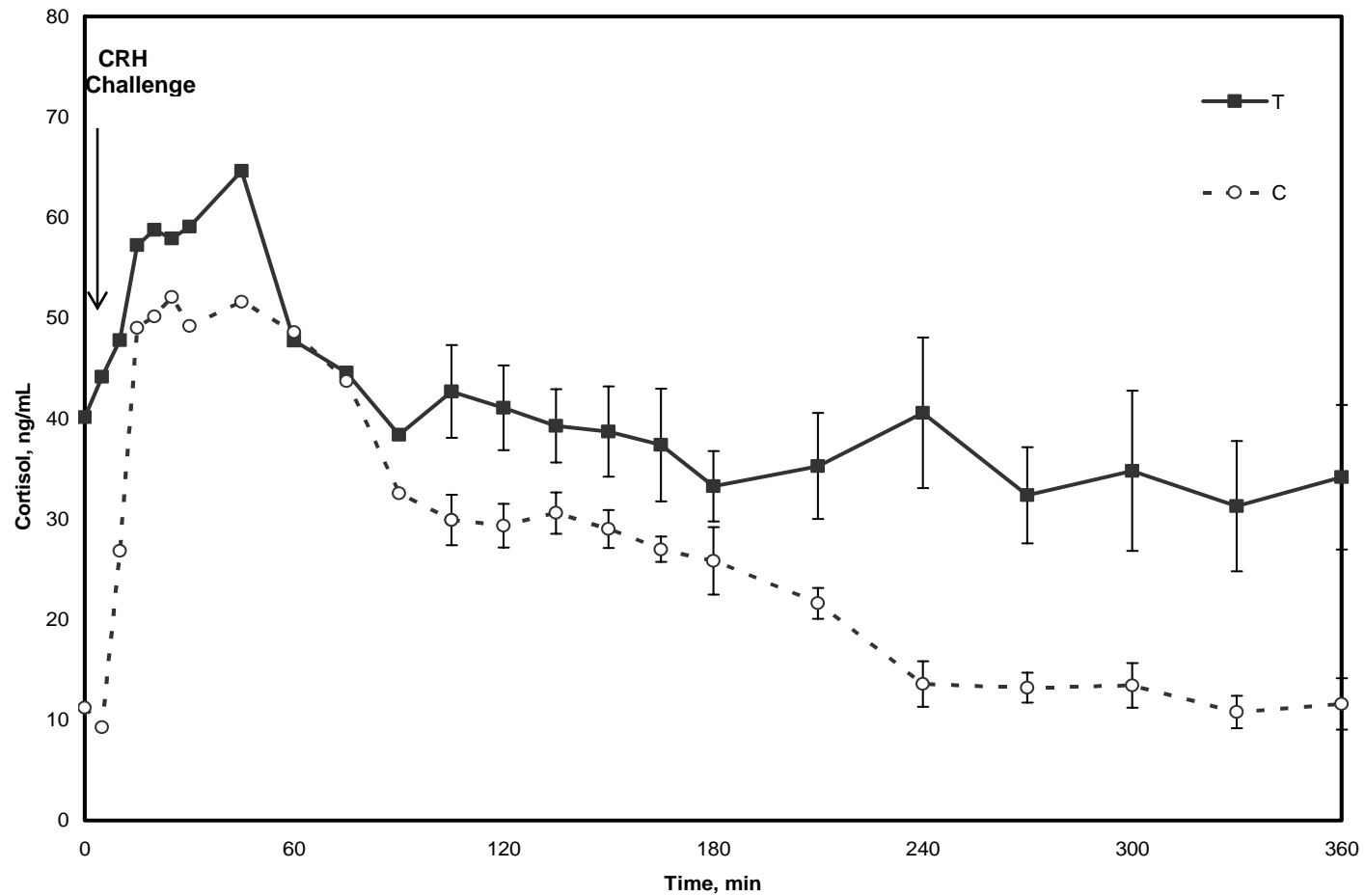


Figure 12. Mean cortisol concentrations over RESP period for both calm (open circles) and temperamental (solid squares) heifers. CRH administered at Time 0. Standard error bars partially omitted to enhance clarity.

While circulating concentrations of cortisol remained higher in the temperamental cattle throughout the entire post-challenge period, adrenal response, considered as a deviation from baseline function, was greatly suppressed in these heifers.

These data suggest a relationship between cattle temperament and functional characteristics of the HPA axis. Due to the initial stress of handling both pituitary and adrenal responses were greater in the temperamental heifers than in the calm heifers, as indicated by analysis of area under the hormone response curves. Following pituitary stimulation with exogenous CRH a suppression of both pituitary ACTH and adrenal CS response was observed. This suppression can be explained as a function of inhibition of HPA axis via feedback mechanisms. Circulating concentrations of CS can exhibit negative feedback upon the HPA axis at the hypothalamic, pituitary, and adrenal levels and can do so with a great degree of temporal variation (Dallman, 1992). The question remains as to whether these observations reflect the effects of a prior acute stressor (i.e., human handling) or a state of chronic stress which can be interpreted by the increased basal cortisol concentrations.

GENERAL CONCLUSIONS

Exit velocity is an effective objective method for temperament assessment in cattle. Temperament measured by EV correlates with other more common temperament assessment methodologies such as pen scores and chute scores. Our study demonstrates the reliability and repeatability of EV measures of temperament over time. The same can not be said for cattle temperament assessed with either pen scores or chute scores. In addition, positive correlations between EV and circulating concentrations of CS demonstrate the ability for EV to gauge bovine HPA axis responsiveness to handling and novel situations. Thus EV can be utilized to characterize individuals within a herd that will exhibit greater stress responses to the handling associated with typical management practices. Selection for cattle with calmer temperaments based on slower exit velocities, could improve herd continuity as well as general animal welfare.

Results from this investigation have demonstrated not only an influence of cattle temperament on stress responses to handling, but also observed a relationship between temperament and basal adrenal function. Animals of poor temperament were shown to have increased baseline concentrations of CS when compared to the herd mates of calmer temperament. Although increased circulating concentrations of CS have been attributed to a plethora of negative physiologic consequences in cattle and may be an explanation why similar detriments are associated with poor temperament, I would be inclined to think otherwise. Typically, increased concentrations of CS, in response to stressors, have been linked to negative consequences. As adaptation is characteristic to

mechanisms of stress and the HPA axis, one could not comment on whether constantly elevated glucocorticoid concentrations would still elicit responses at target tissues.

In addition to increased basal concentrations of CS, reduced responsiveness to pharmacological challenges with exogenous ACTH and CRH were also observed in cattle with excitable temperament. GC inhibition of subsequent stress responses, at the levels of both the hypothalamus and the pituitary has been well documented. Since a relative hypercortisolism was observed in the temperamental cattle, the cause of decreased responsiveness could just be due to elevated CS and increased negative inhibition and not something inherent with poor temperament. Additional investigation is needed in order to clarify the questions that resulted from this study.

LITERATURE CITED

- Bell, P. H., K. S. Howard, R. G. Shepherd, B. M. Finn, and J. H. Meisenhelder. 1956. Studies with corticotrophs. II. Pepsin degradation of β -corticotropin. *J. Am. Chem. Soc.* 78:5059-5066.
- Breuer, K., P. H. Hemsworth, J. L. Barnett, L. R. Matthews, and G.J. Coleman. 2000. Behavioural response to humans and the productivity of commercial dairy cows. *Appl. Anim. Behav. Sci.* 66:273-88.
- Burrow, H. M. 1997. Measurements of temperament and their relationships with performance traits of beef cattle. *Animal Breeding Abstracts* 65:477-495.
- Burrow, H. M. and R. D. Dillon. 1997. Relationships between temperament and growth in a feedlot and commercial carcass traits of *Bos indicus* crossbreds. *Aust. J. Exp. Agr.* 37:407-411.
- Burrow, H. M. and K. C. Prayaga. 2004. Correlated responses in productive and adaptive traits and temperament following selection for growth and heat resistance in tropical beef cattle. *Livest. Prod. Sci.* 86:143-161.
- Burrow, H. M., G. W. Seifert, and N. J. Corbet. 1988. A new technique for measuring temperament in cattle. *Proc. Aust. Soc. Anim. Prod.* 17:154-157.
- Cameron, E.H.D., T. Jones, D. Jones, A.B.M. Anderson, and K. Griffiths. 1969. Further studies on the relationship between C₁₉- and C₂₁-steroid synthesis in the human adrenal gland. *J. Endocrinol.* 45: 215-230.
- Cannon, W.B.. 1932. *The Wisdom of the Body*. W.W. Norton. New York, NY.
- Childs, G. V. 1992. Structure-function correlates in the corticotropes of the anterior pituitary. *Front. Neuroendocrinol.* 13:271-317.
- Dallman, M.F., S.F. Akana, K.A. Scribner, M.J. Bradbury, C.D. Walker, A.M. Strack, and C.S. Cascio. 1992. Stress, feedback and facilitation in the hypothalamo-pituitary-adrenal axis. *J. Neuroendocrinol.* 4:517-26.
- Dallman, M. F., A. M. Strack, S. F. Akana, M. J. Bradbury, E. S. Hanson, K. A. Scribner, and M. Smith. 1993. Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. *Front. Neuroendocrinol.* 14:303-347.
- Dickson, D. P., G. R. Barr, L. P. Johnson, and D. A. Wieckert. 1970. Social dominance and temperament of Holstein cows. *J. Dairy Sci.* 53:904-907.

- Drugociu, G., L. Runceanu, R. Nicorici, V. Hritcu, and S. Pascal. 1977. Nervous typology of cows as a determining factor of sexual productive behaviour. *Anim. Breed. Abstr.* 45:1262. (Abstr.)
- Espiner, E. A., C. A. Jensen, and D. S. Hart. 1972. Dynamics of adrenal response to sustained local ACTH infusions in conscious sheep. *Am. J. Physiol.* 222:570-577.
- Exton, J. H. 1987. Mechanisms of hormonal regulation of hepatic glucose metabolism. *Diabetes Metab. Rev.* 3:163-183.
- Fell, L. R., I. G. Colditz, K. H. Walker, and D. L. Watson. 1999. Associations between temperament, performance and immune function in cattle entering a commercial feedlot. *Aust. J. Exp. Agr.* 39:795-802.
- Fisher, A. D., G. A. Verkerk, C. J. Morrow, and L. R. Matthews. 2002. The effect of feed restriction and lying deprivation on pituitary-adrenal axis regulation in lactating cows. *Livest. Prod. Sci.* 73:255-263.
- Fordyce, G., J. R. Wythes, W. R. Shorthose, D. W. Underwood, and R. K. Shepherd. 1988. Cattle temperaments in extensive beef herds in northern Queensland. 2: Effect of temperament on carcass and meat quality. *Aust. J. Exp. Agr.* 28:689-693.
- Friend, T. H., C. E. Polan, F. C. Gwazdauskas, and C. W. Heald. 1977. Adrenal glucocorticoid response to exogenous adrenocorticotropin mediated by density and social disruption in lactating cows. *J. Dairy Sci.* 60:1958-1963.
- Grandin, T. 1993. Behavioral agitation during handling of cattle is persistent over time. *Appl. Anim. Behav. Sci.* 36:1-9.
- Griffiths, K., J.K. Grant, and T. Symington. 1963. A biochemical investigation of the functional zonation of the adrenal cortex in man. *J. Clin. Endocrinol. Metab.* 23:776-785.
- Gupta, S., B. Earley, S.T.L. Ting, N. Leonard, and M.A. Crowe. 2004. Technical Note: Effect of corticotropin-releasing hormone on adrenocorticotropin hormone and cortisol in steers. *J. Anim. Sci.* 82:1952-1956.
- Hafez, E. S. E. and D. R. Lindsay. 1965. Behavioral responses in farm animals and their relevance to research techniques. *Animal Breeding Abstracts* 33:1-16.

- Hammond, A. C., T. A. Olson, C. C. Chase Jr, E. J. Bowers, R. D. Randel, C. N. Murphy, D. W. Vogt, and A. Tewolde. 1996. Heat tolerance in two tropically adapted *Bos taurus* breeds, Senepol and Romosinuano, compared with Brahman, Angus, and Hereford cattle in Florida. *J. Anim. Sci.* 74:295-303.
- Harris, G. 1948. Neural control of the pituitary gland. *Physiol. Rev.* 28:139-179.
- Hechter, O. 1949. Corticosteroid release from the isolated adrenal gland. *Fed. Proc.* 8:70-71.
- Hench, P. S., E. C. Kendall, C. H. Slocumb, and H. F. Polley. 1949. The effect of a hormone of the adrenal cortex (17-hydroxy-11-dehydrocorticosterone: compound E) and of pituitary adrenocorticotrophic hormone on rheumatoid arthritis. *Proc. Mayo Clinic* 24:181-197.
- Ingle, D. J. and E. C. Kendall. 1937. Atrophy of the adrenal cortex of the rat produced by administration of large amounts of cortin. *Science* 86:245.
- Kaplan, N., and F.C. Bartter. 1962. The effect of ACTH, renin, angiotensin II, and various precursors on biosynthesis of aldosterone by adrenal slices. *J. Clin. Invest.* 41:715-724.
- Kendall, E. C. 1949. The chemistry and partial synthesis of adrenal steroids. *Ann. NY Acad. Sci.* 50:540-547.
- Lacasa, D., B. Agli, and Y. Giudicelli. 1988. Permissive action of glucocorticoids on catecholamine-induced lipolysis: direct in vitro effects on the fat cell beta-adrenoreceptor-coupled-adenylate cyclase system. *Biochem. Biophys. Res. Commun.* 153:489-497.
- Lacourt, A. and P. V. Tarrant. 1985. Glycogen depletion patterns in myofibers of cattle during stress. *Meat Sci.* 15:85-100.
- Laugero, K. D. and G. P. Moberg. 2000. Energetic response to repeated restraint stress in rapidly growing mice. *Am. J. Physiol. - Endoc. M.* 279:E33-E43.
- Lay, D. C., T. H. Friend, R. D. Randel, O. C. Jenkins, D. A. Neuendorff, G. M. Kapp, and D. M. Bushong. 1996. Adrenocorticotrophic hormone dose response and some physiological effects of transportation on pregnant Brahman cattle. *J. Anim. Sci.* 74:1806-1811.
- Liu, J. H., P. Muse, P. Contreras, D. Gibbs, W. Vale, J. Rivier, and S. S. Yen. 1983. Augmentation of ACTH-releasing activity of synthetic corticotropin-releasing factor (CRF) by vasopressin in women. *J. Clin. Endocrinol. Metab.* 57:1087-1089.

- Long, C., B. Katzin, and E. Fry. 1940. The adrenal cortex and carbohydrate metabolism. *Endocrinol.* 26:309-344.
- Mains, R. E., B. A. Eipper, and N. Ling. 1977. Common precursor to corticotropins and endorphins. *Proc. Natl. Acad. Sci. U. S. A* 74:3014-3018.
- Martini, L. and C. Morpurgo. 1955. Neurohumoral control of the release of adrenocorticotrophic hormone. *Nature* 175:1127.
- Mason, H. L. 1964. Only the best is good enough. *J. Clin. Endocrinol. Metab.* 24:1214-1218.
- Moberg, G. P. 1999. When does stress become distress? *Lab Animal* 28:22-26.
- Munck, A. 1971. Glucocorticoid inhibition of glucose uptake by peripheral tissues: old and new evidence, molecular mechanisms, and physiological significance. *Perspect. Biol. Med.* 14:265-269.
- Munck, A., P. M. Guyre, and N. J. Holbrook. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr. Rev.* 5:25-44.
- Nishikawa, T., H. Sasano, M. Omura, and S. Suematsu. 1996. Regulation of expression of the steroidogenic acute regulatory (StAR) protein by ACTH in bovine adrenal fasciculata cells. *Biochem. Biophys. Res. Commun.* 223:12-18.
- O'Bleness, G.V., Van Vleck, L.D. and Henderson, C.R., 1960. Heritabilities of some type appraisal traits and their genetic and phenotypic correlations with production. *J. Dairy Sci.*, 43: 1490-1498.
- Obst, J. M. 1974. The relationship between plasma corticoid and growth rate in cattle. *Proc. Aust. Soc. Anim. Prod.* 10:41-44.
- Pacak, K., M. Palkovits, G. Yadid, R. Kvetnansky, I. J. Kopin, and D. S. Goldstein. 1998. Heterogeneous neurochemical responses to different stressors: a test of Selye's doctrine of nonspecificity. *Am. J. Physiol. - Reg. I.* 44:R1247-R1255.
- Petherick, J. C., R. G. Holroyd, V. J. Doogan, and B. K. Venus. 2002. Productivity, carcass and meat quality of lot-fed *Bos indicus* cross steers grouped according to temperament. *Aust. J. Exp. Agr.* 42:389-398.
- Petherick, J. C., R. G. Holroyd, and A. J. Swain. 2003. Performance of lot-fed *Bos indicus* steers exposed to aspects of a feedlot environment before lot-feeding. *Aust. J. Exp. Agr.* 43:1181-1191.
- Pfiffener, J.J. 1942. The adrenal cortical hormones. *Advan. Enzymol.* 2:325-356.

- Pilkis, S. J. and D. K. Granner. 1992. Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu. Rev. Physiol.* 54:885-909.
- Pohorecky, L. A. and R. J. Wurtman. 1971. Adrenocortical control of epinephrine synthesis. *Pharmacol. Rev.* 23:1-35.
- Pounden, W. D. and J. G. Firebaugh. 1956. Effects of nervousness on conception during artificial insemination. *Vet. Med.* 51:469-470.
- Purcell, D., C. W. Arave, and J. L. Walters. 1988. Relationship of three measures of behavior to milk production. *Appl. Anim. Behav. Sci.* 21:307-313.
- Purchas, R. W., R. A. Barton, and A. H. Kirton. 1980. Relationships of circulating cortisol-levels with growth-rate and meat tenderness of cattle and sheep. *Aust. J. Agr. Res.* 31:221-232.
- Riniker, B., P. Sieber, and W. Rittel. 1972. Revised amino-acid sequences for porcine and human adrenocorticotrophic hormone. *Nature New Biol.* 253:114-115.
- Royce, P. C. and G. Sayers. 1960. Purification of hypothalamic corticotropin releasing factor. *Proc. Soc. Exptl. Biol. Med.* 103:447-450.
- Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* 21:55-89.
- Scott, J. P. and E. Fredericson. 1951. The causes of fighting in mice and rats. *Physiol. Zool.* 24:273-309.
- Selye, H. 1936. A syndrome produced by diverse nocuous agents. *Nature* 138:32.
- Selye, H. 1956. *The Stress of Life*. Revised edition. McGraw-Hill, New York.
- Selye, H. 1971. Hormones and resistance. *J. Pharm. Sci.* 60:1-28.
- Selye, H. 1973. The evolution of the stress concept. *Am. Sci.* 61: 692-699.
- Smith, P. E. 1930. Hypophsectomy and a replacement therapy in the rat. *Am. J. Anat.* 45:205-273.
- Stachenko, J., and C.J.P. Giroud. 1959. Functional zonation of the adrenal cortex: site of ACTH action. *Endocrinol.* 64:743-752.
- Stahringer, R. C., R. D. Randel, and D. A. Neuendorff. 1990. Effects of naloxone and animal temperament on serum luteinizing-hormone and cortisol concentrations in seasonally anestrous Brahman heifers. *Theriogenology* 34:393-406.

- Stenzel-Poore, M., W. W. Vale, and C. Rivier. 1993. Relationship between antigen-induced immune stimulation and activation of the hypothalamic-pituitary-adrenal axis in the rat. *Endocrinol.* 132:1313-1318.
- Stricklin, W. R. and C. C. Kautzscanavy. 1984. The role of behavior in cattle production - A review of research. *Appl. Anim. Ethol.* 11:359-390.
- Sydnor, K.L., and G. Sayers. 1954. Blood and pituitary ACTH in intact and adrenalectomized rats after stress. *Endocrinol.* 55:621-636.
- Trenkle, A., and D.G. Topel. 1978. Relationships of some endocrine measurements to growth and carcass composition of cattle. *J. Anim. Sci.* 46:1604-1609.
- Tulloh, N. M. 1961. Behaviour of cattle in yards. II. A study in temperament. *Anim. Behav.* 9:25-30.
- Turnbow, M. A., S. R. Keller, K. M. Rice, and C. W. Garner. 1994. Dexamethasone down-regulation of insulin-receptor substrate-1 in 3T3-L1 adipocytes. *J. Biol. Chem.* 269:2516-2520.
- Vale, W., J. Spiess, C. Rivier, and J. Rivier. 1981. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 213:1394-1397.
- Veissier, I., C.G. van Reenen, S. Andanson, and I.E. Leushuis. 1999. Adrenocorticotrophic hormone and cortisol in calves alter corticotropin-releasing hormona. *J. Anim. Sci.* 77:2047-2053.
- Voisinet, B. D., T. Grandin, S. F. OConnor, J. D. Tatum, and M. J. Deesing. 1997a. Bos indicus cross feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. *Meat Sci.* 46:367-377.
- Voisinet, B. D., T. Grandin, J. D. Tatum, S. F. OConnor, and J. J. Struthers. 1997b. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. *J. Anim. Sci.* 75:892-896.
- Willard, S.T., J.A. Carroll, R.D. Randel, P.G. Harms and T.H. Welsh, Jr. 1995a. Regulation of adrenocorticotropin secretion in vitro by anterior pituitary corticotrophs from Fallow deer (*Dama dama*). *Domest. Anim. Endocrinol.* 12:283-92.
- Willard, S.T., J.A. Carroll, R.D. Randel, and T.H. Welsh, Jr. 1995b. In vitro cell culture and adrenocorticotropin secretion by Indian blackbuck antelope (*Antilope cervicapra*) anterior pituitary glands collected under field conditions. *J. Zoo Wildl. Med.* 26:252-259.

Zavy, M. T., P. E. Juniewicz, W. A. Phillips, and D. L. VonTungeln. 1992. Effect of initial restraint, weaning, and transport stress on baseline and ACTH-stimulated cortisol responses in beef calves of different genotypes. *Am. J. Vet. Res.* 53:551-557.

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- Curley, Jr. K.O., D.A. Neuendorff, A.W. Lewis, and R.D. Randel. 2003.
Interrelationship between various measurements of temperament in Brahman cows and their Hereford-sired calves. *J. Anim. Sci.* 81(Suppl. 1): 120. (Abstract #476).
- Curley, Jr. K.O., D.A. Neuendorff, A.W. Lewis, J.J. Cleere, T.H. Welsh, Jr., and R.D. Randel. 2004. Effects of temperament on stress indicators in Brahman heifers. *J. Anim. Sci.* 82(Suppl. 1): 459-60. (Abstract #924).
- Curley, Jr. K.O., D.A. Neuendorff, A.W. Lewis, J.J. Cleere, T.H. Welsh, Jr., and R.D. Randel. 2004. Relationship between animal temperament and stress physiology in Brahman cows. *J. Anim. Sci.* 82(Suppl. 2): 21. (Abstract #84).
- Curley, Jr. K.O., D.A. Neuendorff, A.W. Lewis, J.J. Cleere, T.H. Welsh, Jr., and R.D. Randel. 2004. Temperament alters adrenal response to exogenous ACTH in Brahman heifers. *J. Anim. Sci.* 82(Suppl. 1): 260. (Abstract #433).