Theriogenology 89 (2017) 140–145

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Relationships among temperament, acute and chronic cortisol and testosterone concentrations, and breeding soundness during performance testing of Angus bulls

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ARTICLE INFO

Article history:
Received 19 July 2016
Received in revised form 4 October 2016
Accepted 22 October 2016

Keywords:
Bull
Breeding soundness
Cortisol
Temperament
Testosterone

ABSTRACT

The aim of this study was to examine relationships among temperament, endocrinology, and reproductive parameters of bulls enrolled in an 84-day performance test. Angus bulls (n = 60) were housed in six pens grouped by age and weight. Pen scores (PS; 1 = docile to 5 = very aggressive) were assigned on Days −1, 27, 55, and 83 of the performance test. On the following day, blood and hair samples were collected, and body weight (BW) and exit velocity (EV) were recorded. Bulls were split into two categories based on; Day −1 PS (PScalm = PS 1 or 2; PSexcitable = PS 3 or 4) and Day 0 EV (EVcalm = slowest 20 bulls; EVexcitable = fastest 20 bulls). Cortisol and testosterone concentrations in serum and hair did not differ ($P > 0.10$) between PS or EV temperament categories. Sampling day differences ($P < 0.01$) occurred for serum testosterone, hair cortisol, and hair testosterone concentration; however, serum cortisol concentration did not differ ($P > 0.10$) over the sampling days. Serum testosterone concentration increased ($P < 0.01$) from Day 0 to 28, decreased from Day 28 to 56, but Day 84 did not differ from Day 0, 28, or 56. Hair cortisol concentration was greatest ($P < 0.01$) on Day 0, decreased from Day 28 to 56 but did not differ from Day 56 to 84. Hair testosterone concentration was greatest ($P < 0.01$) on Day 0 and remained constant from Day 28 to 84. Bulls categorized as PScalm had a greater ($P < 0.01$) percentage of normal sperm and secondary defects ($P < 0.01$) when compared with PSexcitable bulls. However, EVcalm bulls had fewer ($P < 0.01$) primary defects but more ($P < 0.01$) secondary defects than EVexcitable bulls. In conclusion, bulls exhibited physiological evidence of acclimation during the test as indicated by a reduction in hair cortisol concentration. In addition, the ability of the bulls to acclimate while residing at the testing center may have contributed to little differences observed during the breeding soundness examination portion of the performance test.

1. Introduction

Cattle excitability has been linked to poor performance and can bolster a stimulatory effect on the hypothalamic-pituitary-adrenal axis [1,2]. For instance, excitable cattle, defined by pen score (PS) and exit velocity (EV), have greater circulating cortisol concentrations when compared with calm cattle [1]. Corticosteroids, endogenous or administered, reduce LH and testosterone production and increase spermatic defects [3–5]. However, testosterone production stimulates the interaction between vasopressin and the ventrolateral hypothalamus and induces aggressive behavior in hamsters [6]; but, to our knowledge, no studies

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http://dx.doi.org/10.1016/j.theriogenology.2016.10.019

have examined the effect of testosterone concentration on cattle temperament.

Steroid hormones accrue in hair via vascular supply to the hair follicle, sweat, and sebaceous gland secretions [7]. In addition, hair cortisol concentration has been linked to the activation of the hypothalamic-pituitary-adrenal axis [8]. This response was exhibited by an increase in both circulating and hair cortisol concentrations after adrenocorticotropic hormone challenge in dairy cattle [8]. Previous research has also indicated a positive correlation ($r = 0.395$) between serum and hair testosterone concentration in men [9], and that hair hormone analysis is repeatable [10]. Thus, hair has been used as a noninvasive method to determine concentrations of cortisol and testosterone and may reflect chronic circulating levels in cattle [8,11,12]. To date, we are unaware of other studies conducted to examine bovine hair cortisol and testosterone concentration as it relates to temperament and breeding soundness in a bull performance test. However, we hypothesize that excitable bulls enrolled in an 84-day performance test have lower concentrations of testosterone and higher incidences of sperm abnormalities than calm bulls due to greater cortisol concentrations.

Thus, our aim was to examine relationships among temperament, acute and chronic cortisol, and testosterone concentrations, and breeding soundness in bulls examined over an 84-day period. Furthermore, we examined physiological responses to further explain the results which we previously reported that indicated that bulls deemed excitable, in an 84-day performance test, habituate, and become less excitable over time [13].

2. Materials and methods

2.1. Animal selection and housing

All animal procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee. Bull selection and housing was the same as that reported by Lockwood et al. [13]. Briefly, consigned Black Angus bulls ($n = 60$; 263 ± 36 days of age; 345.3 ± 45.4 kg body weight [BW]) were reared in pens (8–12 bulls/pen) by age and BW. All bulls received ad libitum access to pelleted feed, hay, and water and were provided a 14-day habituation period before the start of the performance test (Day 0).

2.2. Temperament

Pen scores were assigned based on the reactivity of the bull while being approached by the observer on Days −1, 27, 55, and 83. Pen score criteria were as follows: 1 = docile, lets observer approach closely and walks slowly; 2 = runs along fence when observer approaches and is standoffish toward observer; 3 = runs along fence, head held up, and runs away from observer when approached; 4 = runs, very cautious of observer, and may run into fences trying to escape; and 5 = very aggressive, easily agitated, and runs into fences and possibly over observer [14,15]. As each bull was released from the squeeze chute on Days 0, 28, 56, and 84, the time to traverse two infrared sensors, located 1.83 m apart, was recorded and EV was calculated as velocity = distance (m)/time (s) [13,16].

2.3. Tissue collection and analysis

Blood (10 mL) was collected in serum vacutainer tubes via coccygeal venipuncture from each bull on Days 0, 28, 56, and 84 between 7 AM and 12 PM. Serum samples were centrifuged at ×930*g* for 15 minutes and aliquoted in two microcentrifuge tubes and stored at −20 °C.

Total serum cortisol concentration (ng/mL) was determined using the RIA procedure of Coat-A-Count Cortisol (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) as performed previously in our laboratory [17]. Detectable limits were 0 to 500 ng/mL with cross-reactivity less than 12% for related steroids. Intra-assay and interassay coefficient of variations (CVs) were 10.1% and 7.1% for low (9.5 ng/mL) and 7.1% and 10.2% for high (44.5 ng/mL) cortisol standards. Total testosterone concentration (ng/mL) was determined using the RIA procedure of Immu-Chem Double Antibody Testosterone (ICN Biomedicals, Inc., Costa Mesa, CA, USA). Detectable limits were 0.1 to 10 ng/mL with cross-reactivity less than 4% for related steroids. Intra-assay and interassay CVs were 5.9% and 13.0% for low (1.7 ng/mL) and 4.2% and 9.0% for high (4.3 ng/mL) testosterone standards. Coefficients of determination (R^2) of the standard curve were greater than 0.99 for both RIA.

Hair samples were collected on Days 0, 28, 56, and 84 using electric clippers (#40 blades) over the same 20×30 cm area located between the tuber ischii and tuber coxae region of each bull. According to the procedure of González-de-la-Vara Mdel et al. [8], clipper blades were cleaned with absolute ethanol between each bull, and clipped hair samples were placed in ziplock plastic bags and stored at room temperature until analyzed for hair cortisol and testosterone concentration.

Hair cleaning procedures were similar to those described previously [18–20]. Each hair sample (200 mg) was weighed and placed into a 15-mL disposable polypropylene tube and washed four times (3 min/wash) with 3-mL isopropanol to remove manure and environmental debris. After washing, samples were dried on weighing paper at room temperature, wrapped in aluminum foil, and stored for later analyses [21,22].

Hair samples (50 mg) were placed in 2-mL reinforced microcentrifuge tubes with four 2.4-mm metal grinding beads (Omni-International Inc., Kennesaw, GA, USA). Hair samples were then ground to a powder at room temperature in an Omni Bead Ruptor 24 Bead Mill Homogenizer in two, 50-second cycles, of 6.95 m/s with a 15-second pause between cycles (Omni-International Inc., Kennesaw, GA, USA, personal communication).

Hair hormone extraction procedures were performed similarly to those previously described [18]. All extraction procedures were performed at room temperature. Each ground hair sample (30 mg) was placed in a glass vial with 3 mL of high-performance liquid chromatography grade methanol and allowed to extract for 24 hours with gentle shaking [21]. Tubes were then centrifuged for 30 minutes at ×3724*g*. Aliquots of the supernatant were pipetted into separate borosilicate tubes for testosterone (100 μL) and

cortisol (2000 μL) analyses and evaporated under a stream of air. Samples were reconstituted in 6.5-μL high-performance liquid chromatography grade methanol and 123.5-μL assay diluent (Salimetrics, State College, PA, USA) before hormone analyses.

Reconstituted hair extraction samples were analyzed according to Salimetrics Salivary Cortisol and Testosterone EIA (Salimetrics, State College, PA, USA) kit procedures as described previously [18,19,23,24]. Detectable limits for the cortisol and testosterone EIA kits were 0.012 to 3.0 μg/dL and 6.1 to 600 pg/mL, respectively. Cortisol EIA intra-assay and interassay CVs were 4.4% and 7.3% for low (7.15 pg/mg) and 4.5% and 6.2% for high (67.60 pg/mg) cortisol standards with a cross-reactivity less than 1% for other steroids. Testosterone EIA intra-assay and interassay CVs were 6.0% and 11.5% for low (2.68 pg/mg) and 8.0% and 8.8% for high (26.19 pg/mg) testosterone standards. The testosterone EIA cross-reactivity for related steroids, dihydrotestosterone and 19-nortestosterone, was 36.4% and 21.02%, respectively. The testosterone EIA cross-reactivity for all other steroids was less than 2%. Coefficients of determination (R^2) of the standard curve were greater than 0.99 for both EIA.

Hair-processing techniques (cut vs. ground) were compared to determine the efficiency of hormone extraction. Cortisol extracted from the ground hair sample was numerically similar to the concentration of cortisol extracted from the cut hair sample (2.60 pg/mg vs. 2.54 pg/mg, respectively). The validation determined little difference between cortisol concentrations from samples that were extracted for 24 hours versus a 48-hour extraction period (2.60 pg/mg vs. 2.99 pg/mg, respectively). Serial dilutions of reconstituted hair samples (1:2, 1:4, and 1:8) showed a linear reduction in cortisol concentration (1.76, 1.04, and 0.59 pg/mg, respectively). Testosterone concentrations from ground hair samples were numerically greater than that measured from samples cut with scissors (15.33 pg/mg vs. 12.73 pg/mg, respectively). Little difference in testosterone concentration was observed between hair samples that were allowed to extract for 24 hours compared with those following a 48 hours extraction period (12.78 pg/mg vs. 13.15 pg/mg, respectively). In addition, serial dilutions of samples (1:2, 1:4, and 1:8) showed a linear reduction in testosterone concentration (5.96, 3.28, and 1.83 pg/mg, respectively).

2.4. Breeding soundness exams

Scrotal circumference was measured on Day −14 and during the breeding soundness examinations (BSEs) on Day 84 of the test period. Breeding soundness examinations were performed by one licensed veterinarian, board-certified in theriogenology, and followed the guidelines of the Society for Theriogenology [25]. Electroejaculation was used to collect semen samples. Primary spermatic defects (i.e., pyriform head, proximal droplet, and so forth) were classified as abnormalities associated with spermatogenesis, whereas secondary defects (i.e., decapitated normal heads, distal midpiece reflex, and so forth) were a result of storage in the epididymis [26].

2.5. Statistical analysis

All statistical methods were performed in SAS 9.3 (SAS Institute, Cary, NC, USA). As described by Lockwood et al. [13], bulls that received a Day −1 PS of 1 or 2 were categorized as calm (PScalm), whereas bulls with a PS of 3 or 4 were categorized as excitable (PSexcitable). In addition, PROC SORT was used to classify bulls as calm or excitable according to Day 0 EV based on the bottom 20 exit velocities (EVcalm) and top 20 exit velocities (EVexcitable).

A mixed model analysis of variance with bull as experimental unit was used to evaluate the effect of Day −1 PS and Day 0 EV on cortisol and testosterone concentrations in both serum and hair and breeding soundness data collected during the 84-day testing period. Pen number was included in the model as a random blocking effect. Day of sampling was included in the model as a repeated measure for variables assessed multiple times throughout the 84-day testing period. Contemporary groups for PS and EV were fixed effects used to evaluate hormone concentration and breeding soundness. Bull age and weight were retained in the model as covariates when significant ($P < 0.05$). Fisher's least significant difference test was used to separate means ($P < 0.05$). Spearman correlations were also performed to examine relationships among variables.

3. Results and discussion

3.1. Relationships between bull temperament and serum and hair hormone concentrations

The present study found no differences in serum and hair cortisol concentrations among calm and excitable bulls characterized by PS and EV (Table 1). Conversely, Curley et al. [1] reported that circulating cortisol concentrations were greater in bulls with faster EV. As we reported earlier, there was a lack of variation in innate temperament of bulls enrolled in the 84-day performance test based on the overall reduction in PS and EV of bulls initially deemed excitable (Day 0 PS = 3.1; Day 84 PS = 2.2; Day 0 EV = 5.3 m/s; Day 84 EV = 1.8 m/s) [13]. In addition, because serum is an acute measure of circulating cortisol concentration, it is possible that the elevation of circulating cortisol concentration in excitable cattle, reported previously by Curley et al. [1], was only an acute response to handling. In cattle, circulating cortisol concentration begins to rise within minutes of restraint [27]; and thus, may be a reflection of the fear response toward handlers and restraint. It is possible that after release from confinement and removal from the presence of the handlers, circulating cortisol in excitable bulls decreases as shown by similar hair cortisol concentrations between PS and EV temperament categories in the present study.

Similar to cortisol, serum and hair testosterone concentration did not vary between EV and PS temperament categories. Thibier and Rolland [3] reported that administration of dexamethasone, a synthetic corticosteroid, caused a reduction in circulating testosterone, but because cortisol concentration was within ranges reported previously for bulls [28] and did not vary between temperament categories, it is not surprising that

Table 1
Mean ± standard error of the mean serum and hair hormone concentrations for bulls deemed either calm or excitable according to initial pen score and exit velocity.[a]

Hormone measured	Day	Temperament assessment method			
		Pen score[b]		Exit velocity[c]	
		Calm (n = 40)	Excitable (n = 20)	Calm (n = 20)	Excitable (n = 20)
Serum cortisol, ng/mL	0	8.51 ± 1.27	10.16 ± 2.11	11.53 ± 2.32	12.43 ± 2.34
	28	7.70 ± 1.26	7.47 ± 1.53	8.39 ± 2.32	13.85 ± 2.34
	56	8.36 ± 1.26	8.66 ± 1.77	11.95 ± 2.32	13.55 ± 2.34
	84	8.24 ± 1.23	12.86 ± 2.63	12.12 ± 2.35	11.55 ± 2.36
Serum testosterone, ng/mL	0	1.74 ± 0.20	1.77 ± 0.28	1.63 ± 0.36	1.05 ± 0.23
	28	2.20 ± 0.20	2.29 ± 0.28	1.95 ± 0.44	1.40 ± 0.31
	56	1.64 ± 0.20	1.82 ± 0.28	1.28 ± 0.29	1.10 ± 0.25
	84	2.00 ± 0.21	2.06 ± 0.28	1.60 ± 0.37	1.41 ± 0.32
Hair cortisol, pg/mg	0	5.45 ± 0.74	5.67 ± 0.86	5.38 ± 0.87	5.97 ± 0.98
	28	3.49 ± 0.47	4.06 ± 0.61	4.20 ± 0.68	3.61 ± 0.59
	56	2.47 ± 0.33	2.63 ± 0.40	2.47 ± 0.40	2.52 ± 0.41
	84	2.49 ± 0.34	2.39 ± 0.36	2.51 ± 0.41	2.48 ± 0.41
Hair testosterone, pg/mg	0	13.09 ± 1.09	11.30 ± 1.16	12.29 ± 1.32	13.29 ± 1.45
	28	9.92 ± 0.83	8.74 ± 0.90	9.69 ± 1.04	10.04 ± 1.08
	56	9.06 ± 0.75	9.09 ± 0.94	9.44 ± 1.01	8.81 ± 0.95
	84	8.74 ± 0.74	8.05 ± 0.86	9.03 ± 1.00	8.22 ± 0.89

[a] Serum and hair hormone concentrations did not differ between pen score and exit velocity temperament categories ($P > 0.10$).
[b] Pen score was assigned on Day −1 based on the reactivity of each bull when approached by a human observer (1 = docile to 5 = very aggressive).
[c] Exit velocity was assessed on Day 0 and is defined as the rate of speed traversing 1.83 m, recorded as meters per second.

differences in testosterone concentration were not observed between temperament categories in our study. Furthermore, the range for serum testosterone concentration (1.05–2.29 ng/mL) for all bulls in the present study fell within the lower portion of the prechallenge range reported by Thibier and Rolland [3]. Thus, it is not surprising that testosterone concentration did not vary between temperament categories.

Sampling day differences ($P < 0.01$) occurred for serum testosterone, hair cortisol, and hair testosterone concentration; however, serum cortisol concentration did not differ ($P > 0.10$) over the sampling days (Table 2). Serum testosterone concentration increased ($P < 0.01$) from Day 0 to 28, decreased from Day 28 to 56, but Day 84 did not differ from Days 0, 28, or 56. The peak in serum testosterone concentration observed on Day 28 may be attributed to bulls reaching puberty. On Day 28 of the study, bulls ranged from approximately 9 to 10.5 months of age, which coincides with the average age of puberty in Angus bulls [29]. Similarly, Rawlings et al. [30] reported that circulating testosterone concentrations in Holstein bulls increased until 11 months of age but decreased by 12 months of age. The reduction in serum testosterone concentration observed on Day 56 in the present study followed a similar pattern as that reported by Rawlings et al. [30].

Table 2
Mean serum and hair hormone concentrations for all bulls (n = 60) over the 84-day testing period.[a,b]

Hormone measured	Day	Concentration ± SEM
Serum cortisol, ng/mL	0	12.07 ± 1.57
	28	11.16 ± 1.56
	56	11.95 ± 1.56
	84	11.91 ± 1.57
Serum testosterone, ng/mL	0	1.76^{2} ± 0.16
	28	2.23^{1} ± 0.16
	56	1.71^{2} ± 0.16
	84	$2.01^{1,2}$ ± 0.16
Hair cortisol, pg/mg	0	5.50^{1} ± 0.76
	28	3.63^{2} ± 0.50
	56	2.52^{3} ± 0.35
	84	2.43^{3} ± 0.34
Hair testosterone, pg/mg	0	12.48^{1} ± 0.87
	28	9.52^{2} ± 0.66
	56	9.09^{2} ± 0.63
	84	8.58^{2} ± 0.61

Means within a hormone sampling method with different superscript numbers (1–3) differ between day of sampling ($P < 0.01$).
Abbreviation: SEM, standard error of the mean.
[a] Serum samples were collected from each bull on Days 0, 28, 56, and 84.
[b] Hair samples were collected from the same 20 × 30 cm region of each bull on Days 0, 28, 56, and 84.

Hair cortisol concentration was greatest ($P < 0.01$) on Day 0, decreased from Day 28 to 56, but concentrations remained constant from Day 56 to 84 (Table 2) and were similar to those previously reported by Moya et al. [31]. As mentioned earlier, the bulls appeared to have habituated between Days 0 and 56 as shown by a reduction in EV and PS in excitable bulls [13]. The decrease in cortisol deposition in the hair shaft further supports that bulls acclimated to the testing center and thus, may have attributed to the lack of temperament variation at the conclusion of the test. Hair testosterone concentration was greatest ($P < 0.01$) on Day 0 but were stable from Day 28 to 84 (Table 2) and were similar to concentrations previously reported in young bulls [32]. We attribute the elevated hormone concentrations found in the hair samples collected on Day 0 of study to be associated with hormone accumulation before the start of the test, as hair samples were longer on Day 0 when compared to those collected on all other sampling days. Interestingly, the new hair growth that occurred after each clipping on Days 0, 28, and 56 possessed similar concentrations of testosterone, indicating that testosterone was deposited into the hair shaft at a repeatable rate after the initial hair clipping.

Table 3
Mean ± SEM breeding soundness examination variables measured on completion of the performance test (Day 84) for bulls deemed either calm or excitable according to initial pen score and exit velocity contemporary groups.

Reproductive variable[a]	Temperament assessment method			
	Pen score[b]		Exit velocity[c]	
	Calm (n = 40)	Excitable (n = 20)	Calm (n = 20)	Excitable (n = 20)
Normal sperm, %	63.62[1] ± 3.11	56.53[2] ± 3.49	65.10 ± 3.33	61.85 ± 3.23
Primary defects, %	24.20 ± 1.97	27.83 ± 2.55	21.36[2] ± 2.15	26.78[1] ± 2.61
Secondary defects, %	5.51[1] ± 0.55	3.92[2] ± 0.61	8.08[1] ± 0.59	3.80[2] ± 0.57

Means within a row within a temperament assessment method with different superscript numbers (1 and 2) differ (P < 0.01).
Abbreviation: SEM, standard error of the mean.
[a] Reproductive variables were measured during breeding soundness examinations on Day 84. Semen was collected via electroejaculation.
[b] Pen score was assigned on Day −1 based on the reactivity of each bull when approached by a human observer (1 = docile to 5 = very aggressive).
[c] Exit velocity was assessed on Day 0 and is defined as the rate of speed traversing 1.83 m, recorded as meters per second.

3.2. Temperament and reproductive parameters

Scrotal circumference did not differ (P > 0.10) between PScalm or PSexcitable bulls on Days −14 (26.57 cm ± 0.51 vs. 26.89 cm ± 0.66, respectively) or 84 (35.57 cm ± 0.51 vs. 35.01 cm ± 0.67, respectively). In addition, scrotal circumference did not differ (P > 0.10) between EVcalm and EVexcitable bulls on Days −14 (27.34 cm ± 0.71 vs. 27.03 cm ± 0.72, respectively) or 84 (35.76 cm ± 0.72 vs. 35.33 cm ± 0.72, respectively). However, overall, scrotal circumference measured on Day 84 was greater (P < 0.001) than that measured on Day −14 (35.49 cm ± 0.64 vs. 26.40 cm ± 0.48, respectively). Although Burrow [33] previously reported a weak positive correlation between exit velocity and scrotal circumference, we attribute our results to the lack of innate temperament variation of bulls in the present study. In regards to the BSE data collected on Day 84, some differences (P < 0.01) in sperm characteristics were observed between PS and EV categories (Table 3). Bulls categorized as PScalm had a greater (P < 0.01) percentage of normal sperm and secondary defects than PSexcitable bulls. Furthermore, EVcalm bulls had a greater (P < 0.01) percentage of secondary defects but possessed fewer (P < 0.01) primary defects when compared to EVexcitable bulls. However, because the total number of primary and secondary spermatic defects typically equates to approximately 30%, which is the accepted allowance for sperm morphological defects [34], temperament does not appear to impact the breeding potential of bulls in the present study.

3.3. Relationships between serum and hair hormone concentrations

No relationship (−0.30 < r < 0.30) was found between serum and hair cortisol concentration on any day of test. Previously, Hopster et al. [27] reported that serum cortisol concentrations increased within minutes of blood collection but plateaued around 30 minutes after the first blood sample collection. We suspect that the circadian release of cortisol, its alterations by acute stressors, the blood sampling time frame, and hair growth lag time were likely the reasons and there was no relationship observed between acute and chronic steroid hormone assessment methods [27,35].

Serum and hair testosterone concentrations were correlated on Days 0 and 28 (r = 0.30 and 0.34, respectively; P < 0.001) but showed no relationship (−0.30 < r < 0.30) on Days 56 and 84. Previously, Yang et al. [9] reported a positive correlation (r = 0.40) between serum and hair testosterone in adult men. Differences in our results in comparison to those reported by Yang et al. [9] may be attributed to species differences or as a result of varying stages of puberty. Furthermore, circulating hormone concentrations can fluctuate rapidly and may not be reflected in hair samples, since hormone levels measured in hair represent an accumulation over time [36].

Day 84 serum cortisol concentration was unrelated (−0.30 < r < 0.30) to percentage of normal sperm and secondary spermatic defects but was positively correlated (r = 0.36; P < 0.001) with primary defects. Furthermore, no relationships (−0.30 < r < 0.30) were found between cortisol measured in hair and the BSE data. These results were unsurprising because circulating cortisol concentrations for the junior bulls in the present study were less than the basal concentration of 15 ng/mL reported previously for mature Angus bulls [37], and did not indicate that bulls were in a stressful state.

3.4. Conclusions

In summary, although cortisol and testosterone concentrations in both serum and hair were not different between temperament categories as defined in our study, the decrease in hair cortisol concentration following Day 0 suggested physiological evidence of acclimation that agreed with the behavioral habituation reported by Lockwood et al. [13]. In addition, after the initial hair clipping, hair testosterone proved to be deposited equivalently between 28-day sampling points. Furthermore, because the bulls in the performance test will likely reside within a breeding program, it is possible that they were preselected for disposition by the consignor before arriving at the testing center. The ability of the bulls to acclimate to the testing center may have also contributed to little differences observed during the BSE portion of the performance test.

Acknowledgments

This research is published with the approval of the Dean of UTIA AgResearch and supported by State and Hatch Funds allocated to the college. This research did not receive

any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Appreciation is extended to Barbara Gillespie and the personnel of the University of Tennessee's Middle Tennessee Research and Education Center for assistance with the conduction of this study.

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