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

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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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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