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Effects of temperament and acclimation to handling on reproductive performance of *Bos taurus* beef females[1]

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ABSTRACT: Two experiments evaluated the effects of temperament and acclimation to handling on reproductive performance of *Bos taurus* beef females. In Exp. 1, 433 multiparous, lactating Angus × Hereford cows were sampled for blood and evaluated for temperament before the breeding season. Cow temperament was assessed by chute score and exit velocity. Chute score was assessed on a 5-point scale according to behavioral responses during chute restraining. Exit score was calculated by dividing exit velocity into quintiles and assigning cows with a score from 1 to 5 (1 = slowest, 5 = fastest cows). Temperament score was calculated by averaging chute and exit scores. Cows were classified for temperament type according to temperament score (≤ 3 = adequate, > 3 = aggressive). Plasma cortisol concentrations were greater ($P < 0.01$) in cows with aggressive vs. adequate temperament. Cows with aggressive temperament had reduced ($P \leq 0.05$) pregnancy and calving rate and tended to have reduced ($P = 0.09$) weaning rate compared with cows with adequate temperament. Hence, kilogram of calf born per cow was reduced ($P = 0.05$) and kilogram of calf weaned per cow tended to be reduced ($P = 0.08$) in aggressive cows. In Exp. 2, 88 Angus × Hereford heifers (initial age = 206 ± 2 d) were weighed (d 0 and 10) and evaluated for temperament score (d 10). On d 11, heifers were ranked by these variables and assigned to receive or not (control) an acclimation treatment. Acclimated heifers were processed through a handling facility 3 times weekly for 4 wk (d 11 to 39; Mondays, Wednesdays, and Fridays), whereas control heifers remained undisturbed on pasture. Heifer puberty status, evaluated via plasma progesterone concentrations, was assessed on d 0 and 10, d 40 and 50, 70 and 80, 100 and 110, 130 and 140, 160 and 170, and 190 and 200. Blood samples collected on d 10 and 40 were also analyzed for plasma concentrations of cortisol and haptoglobin. Temperament score was assessed again on d 40 and d 200. Acclimated heifers had reduced ($P = 0.01$) concentrations of cortisol and haptoglobin on d 40 and reduced ($P = 0.02$) exit velocity on d 200 compared with control heifers. Puberty was hastened in acclimated heifers compared with control ($P = 0.01$). Results from this study indicate that *B. taurus* beef cows with aggressive temperament have impaired reproductive performance compared with cohorts with adequate temperament, whereas acclimation to human handling after weaning hastens reproductive development of replacement heifers.

Key words: acclimation, *Bos taurus*, handling, reproduction, temperament

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INTRODUCTION

Reproductive efficiency of cow-calf herds is optimal when replacement heifers become pubertal as yearlings (Bagley, 1993) and cows become pregnant during the annual breeding season (Rae, 2006). Therefore, recognition of traits that modulate puberty and fertility in beef females is essential for optimal profitability of cow-calf operations. Recently, our research group reported that beef cows with excitable

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temperament had reduced pregnancy rates compared with cohorts with adequate temperament (Cooke et al., 2009a, 2011), whereas acclimation to human handling improved temperament and hastened puberty attainment in heifers (Cooke et al., 2009b). These outcomes were attributed, at least partially, to altered neuroendocrine stress responses associated with temperament that can disrupt the physiological processes required for proper reproductive function (Dobson et al., 2001).

However, the effects of temperament and acclimation to human handling on female reproduction were only evaluated in *Bos indicus*–influenced cattle (Cooke et al., 2009a,b, 2011). Excitable temperament is also detected among *B. taurus* breeds, particularly in young animals and cattle reared in extensive systems (Fordyce et al., 1988; Morris et al., 1994). Therefore, we hypothesized that temperament also impacts reproductive efficiency of range cow-calf operations composed of *B. taurus* cattle. In addition, frequent handling has been shown to improve temperament of young cattle independently of breed type (Krohn et al., 2001; Curley et al., 2006). Hence, we also hypothesized that acclimation to human handling enhances reproductive development of *B. taurus* heifers. To address these hypotheses, Exp. 1 associated temperament, physiological responses, and reproductive performance of Angus × Hereford cows, and Exp. 2 evaluated the effects of acclimation to human handling on temperament, physiological, and reproductive responses of Angus × Hereford heifers.

MATERIALS AND METHODS

All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee.

Experiment 1 was conducted at the Oregon State University Eastern Oregon Agricultural Research Center (**OSU-EOARC**; Burns and Union, OR) from May 2009 to October 2010, whereas Exp. 2 was conducted at the OSU-EOARC (Burns, OR) from October 2009 to August 2011. Animal handling facilities at both locations used a Silencer Chute (Moly Manufacturing, Lorraine, KS) mounted on Avery Weigh-Tronix load cells (Fairmount, MN; readability 0.45 kg).

Experiment 1

Animals. A total of 433 multiparous and lactating Angus × Hereford cows (Burns, n = 239; Union, n = 194) were assigned to the experiment (mean BW = 466 ± 3 kg). Before the annual breeding season, cows from both locations were sampled for blood and evaluated for BCS (Wagner et al., 1988; by the same 2 technicians in both locations) and temperament (Cooke et al., 2011; by the same single technician in both locations). Cows from the Burns location were exposed (beginning June 2009) to mature bulls (age = 5.2 ± 0.3 yr) for a 50-d breeding period (1:18 bull to cow ratio). Cows from the Union location were assigned (beginning April 2009) to an estrus synchronization + timed AI protocol (CO-Synch + controlled internal progesterone-release device; Larson et al., 2006) and were exposed immediately after AI to mature bulls (age = 5.6 ± 0.4 yr) for 50 d (1:24 bull to cow ratio). Cows were inseminated by the same technician with semen from the same bull. All bulls used in this experiment were submitted to and approved by a breeding soundness evaluation (Chenoweth and Ball, 1980) before the breeding season.

All cows from the Burns location were managed on semiarid range pastures (Ganskopp and Bohnert, 2009) from May to November 2009 and on flood meadow pastures harvested for hay the previous summer (Merrill et al., 2008) from December 2009 to April 2010. Pregnancy status was verified by detecting a fetus via rectal palpation by a single licensed veterinarian in December 2009, approximately 120 d after the end of the breeding season. Cows calved between March and April 2010. On May 2010, cow-calf pairs returned to range, and calves were weaned in September 2010. Pregnancy loss was calculated on the basis of pregnancy diagnosis after the breeding season and calving rates. Calf BW was determined within 2 d after birth and at weaning.

All cows from the Union station were managed in mixed-conifer range pastures (Damiran et al., 2003) from July until October 2009 and on mixed-grass pastures harvested for hay the previous summer (Horney et al., 1996) from November 2009 until June 2010. Pregnancy status was verified by detecting a fetus via rectal palpation by a single licensed veterinarian in December 2009, approximately 180 d after the end of the breeding season. Cows calved between February and March 2010. On July 2010, cow-calf pairs returned to mixed-conifer range, and calves were weaned in October 2010. Pregnancy loss was calculated on the basis of pregnancy diagnosis after the breeding season and calving rates. Calf BW was determined within 2 d after birth and at weaning.

Sampling. At the Burns location, blood samples were collected, and BCS and temperament were assessed when cows were restrained and processed for transport to semiarid range pastures. A total of 6 trained technicians participated in the sampling process. At the Union location, these measurements were obtained when cows were restrained for the first GnRH administration of the estrus synchronization protocol. A total of 5 trained technicians participated in the sampling process. Blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin (148 USP units), placed on ice immediately, and centrifuged at 2,400 × *g* for 30 min at room temperature for plasma collection. Plasma was stored at −80°C on the same day of collection. Plasma concentrations of cortisol were determined using a bovine-specific commercial ELISA kit (Endocrine Technologies Inc., Newark, CA). The intra- and interassay CV were 4.9% and 4.0%, respectively.

Individual cow temperament was assessed by chute score and exit velocity as previously described by Cooke et al. (2011). Chute score was assessed by a single technician on the basis of a 5-point scale, where 1 = calm with no movement, 2 = restless movements, 3 = frequent movement with vocalization, 4 = constant movement, vocalization, shaking of the chute, and 5 = violent and continuous struggling. Exit velocity was assessed immediately by determining the speed of the cow exiting the squeeze chute by measuring rate of travel over a 1.9-m distance with an infrared sensor (FarmTek Inc., North Wylie, TX). Further, within location, cows were divided in quintiles according to their exit velocity and were assigned a score from 1 to 5 (exit score; 1 = cows within the slowest quintile, 5 = cows within the fastest quintile). Individual temperament scores were calculated by averaging cow chute score and exit score. Cows were classified according to the final temperament score (temperament type) as adequate temperament (temperament score ≤3) or excitable temperament (temperament score >3).

Statistical Analysis. All data were analyzed using cow as the experimental unit with model statements that contained fixed effects of cow temperament measurement (temperament score or type), location, and the resultant interaction and a random statement that contained the effect of cow (location × temperament measurement). Effects of cow temperament on BCS, plasma cortisol, kilogram of calf born per cow exposed to breeding, and kilogram of calf weaned per cow exposed to breeding were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) with Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Effects of cow temperament on pregnancy rate, pregnancy loss, calving rate, and weaning rate were analyzed using the GLIMMIX procedure of SAS with Satterthwaite approximation. Effects of cow temperament on calf birth weight, weaning age, and weaning BW were also analyzed using the MIXED procedure of SAS with Satterthwaite approximation. These model statements were the same as previously described but contained the effects of cow (location × temperament measurement) and calf sex as random variables. Results are reported as least-squares means and separated using LSD. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to temperament effects if no interactions were significant or according to the greatest-order interaction detected.

Experiment 2

Animals. A total of 88 Angus × Hereford heifers (yr 1, n = 38; yr 2, n = 50) weaned at approximately 6 mo of age were assigned to the experiment. Within 30 d after weaning, heifers were evaluated for BW (d 0 and 10 of the study) and temperament score (d 10 only) as described in Exp. 1. On d 11, heifers were ranked by temperament score and BW and were assigned to receive or not (control) an acclimation treatment. Across year, heifer mean BW and age (±SEM) at the beginning of the experiment were 223 ± 2 kg and 206 ± 2 d, respectively.

Diets. During the entire experiment, heifers were maintained on separate 6-ha meadow foxtail (*Alopecurus pratensis* L.) pastures harvested for hay the previous summer according to treatment. Heifers were rotated between pastures every 4 wk. Heifers from both treatments received meadow foxtail and alfalfa hay at a rate to provide a daily amount of 7.0 and 1.0 kg of DM per heifer, respectively. Water and a commercial mineral and vitamin mix (Cattleman's Choice; Performix Nutrition Systems, Nampa, ID) containing 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 6000 mg/kg Zn, 3200 mg/kg Cu, 65 mg/kg I, 900 mg/kg Mn, 140 mg/kg Se, 136 IU/g of vitamin A, 13 IU/g of vitamin D_3, and 0.05 IU/g of vitamin E were offered for ad libitum consumption throughout the experiment. Hay samples were collected at the beginning of the experiment and were analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) using wet chemistry procedures for concentrations of CP (method 984.13; AOAC, 2006), ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, NY; AOAC, 2006), and NDF (Van Soest et al., 1991; method for use in an Ankom 200 fiber analyzer, Ankom Technology Corp.). Calculations of TDN used the equation proposed by Bath and Marble (1989), whereas NEm and NEg were calculated with the equations proposed by the NRC (1996). Averaged over the 2 yr of study, meadow foxtail and alfalfa hay quality were estimated at (DM basis), respectively, 58% and 63% TDN, 65% and 44% NDF, 33% and 26% ADF, 1.19 and 1.35 Mcal/kg of NEm, 0.62 and 0.78 Mcal/kg of NEg, and 5.2% and 22.0% CP.

Acclimation Procedure. Acclimated heifers were exposed to a handling acclimation process 3 times weekly (Monday, Wednesday, and Friday) for 4 wk (d 11 to 39 of the experiment). The acclimation treatment was applied individually to heifers by processing them through a handling facility, as previously described by Cooke et al. (2009b), by 2 trained technicians. During the first week of acclimation, heifers were individually processed through the handling facility but were not restrained in the squeeze chute. During the second week, heifers were individually processed through the handling facility and were restrained in the squeeze chute for approximately 5 s. On the third and fourth weeks, heifers were similarly processed as in wk 2 but were restrained in the squeeze chute for 30 s. During the initial 3 wk, heifers were allowed to return to their pasture immediately after processing, whereas during the fourth week heifers remained in the handling facility for 1 h, were processed again through the handling facility, and then returned to pasture. For each handling acclimation process, acclimated heifers were gathered in the pasture and obliged to walk to the handling facility, whereas control heifers remained undisturbed on pasture. The total distance traveled by acclimated heifers during each of the acclimation events was approximately 0.6 km (round-trip).

Sampling. Heifer BW and puberty status were assessed on d 0 and 10, 40 and 50, 70 and 80, 100 and 110, 130 and 140, 160 and 170, and 190 and 200. Heifer BW gain was calculated by averaging the values obtained in both 10-d interval assessments. Further, heifer shrunk (after 16 h of feed and water restriction) BW was collected on d 1 and 201 for calculation of heifer ADG during the study. Puberty was assessed via plasma progesterone (**P4**). Heifers were considered pubertal once P4 concentrations greater than 1.0 ng/mL (Perry et al., 1991) were detected within a 10-d interval assessment and consecutively detected in at least 1 sample during the subsequent 10-d interval assessments. Blood samples collected on d 10 and 40 were also analyzed for plasma concentrations of cortisol and haptoglobin. Heifer temperament score was also evaluated on d 40 and 200, as described in Exp. 1.

On d 205, heifers from both treatments were combined into a single pasture, assigned to the estrus synchronization + timed AI described in Exp. 1, and exposed to mature bulls (1:25 bull to heifer ratio) for 48 h following the PG F2α injection of the protocol and for 50 d beginning 12 h after AI. Within each year, heifers were inseminated, and bulls were evaluated for breeding soundness as in Exp. 1. Heifer pregnancy status was verified by detecting a fetus via rectal palpation approximately 200 d after the end of the breeding season in yr 1 and via transrectal ultrasonography (5.0-MHz transducer; 500V, Aloka, Wallingford, CT) 100 d after the end of the breeding season in yr 2.

Blood Analysis. Blood samples were collected and harvested for plasma as in Exp. 1. Concentrations of P4 were determined according to the ELISA procedure described by Galvão et al. (2004). Plasma concentrations of cortisol were determined as in Exp. 1. Plasma concentrations of haptoglobin were determined according to a colorimetric procedure that measures haptoglobin-hemoglobin complexing described by Makimura and Suzuki (1982). Across year, the intra- and interassay CV were, respectively, 7.2% and 5.2% for cortisol, 9.2% and 12.3% for P4, and 5.8% and 1.9% for haptoglobin.

Statistical Analysis. All data were analyzed using heifer as the experimental unit and heifer (treatment × year) as a random variable. Growth, temperament, and physiological data were analyzed using the MIXED procedure of SAS and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement used for analysis of temperament, BW gain, and physiological data contained the fixed effects of treatment, day of the study, year, and the resultant interactions. Physiological data were adjusted covariately to values obtained before acclimation period (d 10). The specified term used in the repeated statement for temperament and BW gain analysis was day, the subject was heifer(treatment × year), and the covariance structure used was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. The model statement used for ADG contained the fixed effect of treatment, year, and the interaction. Puberty and pregnancy data were analyzed using the GLIMMIX procedure of SAS with Satterthwaite approximation. The model statement contained the fixed effects of treatment, year, day of the study (puberty only), and the resultant interactions. Results are reported as least-squares means and separated using LSD. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to treatment effects if no interactions were significant or according to the greatest-order interaction detected.

RESULTS AND DISCUSSION

Experiment 1

Independent of breed type, aggressive cattle have impaired growth (Voisinet et al., 1997b; Nkrumah et al., 2007), health (Fell et al., 1999; Burdick et al., 2010), and carcass quality (Voisinet et al., 1997a; King et al., 2006; Cafe et al., 2011) compared with calm cohorts, demonstrating the importance of cattle temperament to beef production systems. Our research group was the first to report that temperament also has direct implications to reproductive performance of beef females (Cooke et al., 2009a,b, 2011). However, these research studies only evaluated *B. indicus*–influenced cattle, and to our knowledge, the present paper is the first to assess the effects of temperament on reproductive and overall performance of *B. taurus* beef females. On the basis of the temperament evaluation criteria adopted herein, both locations had similar ($P \geq 0.65$; data not shown) mean temperament score of the herd (2.52 vs. 2.48 temperament score for Burns and Union, respectively; SEM =

0.06) and proportion of aggressive animals (25.9% vs. 24.2% of aggressive animals/total animals for Burns and Union, respectively; SEM = 2.9). It is important to note that the goal of the present experiment was to determine if temperament impacts reproduction in *B. taurus* beef females and not to determine the incidence of excitable beef females in range cow-calf operations based on *B. taurus* cattle. The methods and criteria used herein to evaluate cattle for temperament were similar to our previous research efforts with *B. indicus*–influenced cattle (Cooke et al., 2009a,b, 2011) and have the purpose of classifying cattle according to temperament characteristics by using techniques that can be feasibly completed during routine cattle processing (Cooke et al., 2011).

Cattle with aggressive temperament may have reduced feed intake (Nkrumah et al., 2007) and impaired nutrient metabolism (Elsasser et al., 1997; Maciel et al., 2001; Carroll and Forsberg, 2007) compared with cohorts with adequate temperament. Therefore, temperament may indirectly impact reproductive performance of beef females by impairing their nutritional status (Wettemann and Bossis, 2000). However, in the present study, cow BCS did not change according to temperament score ($P = 0.31$; data not shown) and was similar between cows with adequate and aggressive temperaments ($P = 0.17$; Table 1). These results are similar to previous research from our group with *B. indicus*–influenced females (Cooke et al., 2009a, 2011) and indicate that any effects of temperament on reproductive performance are independent of cow nutritional status.

Table 1. Performance, reproductive, and physiological variables of *Bos taurus* beef cows (±SE) according to temperament

Item	Temperament type[1]		
	Adequate (n = 324)	Aggressive (n = 109)	*P*-value
Cow variables[2]			
Cow BCS	4.65 ± 0.02	4.59 ± 0.04	0.17
Plasma cortisol, ng/mL	17.8 ± 0.6	22.7 ± 1.0	<0.01
Pregnancy rate, %	94.6 ± 1.4	88.7 ± 2.4	0.03
Pregnancy loss, %	2.83 ± 0.95	3.74 ± 1.65	0.63
Calving rate, %	91.8 ± 1.6	85.0 ± 2.8	0.04
Calf variables			
Calf birth BW, kg	39.7 ± 2.1	40.0 ± 2.1	0.52
Calf weaning age, d	201 ± 1	203 ± 3	0.45
Calf weaning BW, kg	248 ± 5	247 ± 6	0.71
Cow-calf production variables[3]			
Calf born per cow exposed, kg	36.8 ± 0.7	34.1 ± 1.2	0.05
Calf loss from birth to weaning, %	1.92 ± 0.70	1.06 ± 1.22	0.54
Weaning rate, %	89.9 ± 1.7	83.9 ± 3.0	0.09
Calf weaned per cow exposed, kg	223 ± 4	207 ± 8	0.08

[1]Calculated on the basis of cow temperament score (adequate temperament, temperament score ≤ 3; excitable temperament, temperament score > 3). Temperament score was calculated by averaging cow chute score and exit score. Exit score was calculated by dividing exit velocity results into quintiles and assigning cows with a score from 1 to 5 (exit score: 1 = slowest cows; 5 = fastest cow).

[2]Blood samples were collected and BCS was recorded concurrently with temperament evaluation, before the annual breeding season. Pregnancy loss was calculated on the basis of pregnancy diagnosis after the breeding season and calving rates.

[3]Kilograms of calf born and calf weaned per cow exposed were calculated on the basis of calving rate, weaning rate, and calf BW at birth and weaning.

Plasma cortisol concentrations increased ($P < 0.01$; Figure 1) as temperament score increased, concurring with previous findings from our group (Cooke et al., 2009a,b). Hence, plasma cortisol concentrations were greater ($P < 0.01$) in cows with aggressive temperament compared with cohorts with adequate temperament (Table 1). Temperament score was not associated with any of the reproductive and cow-calf performance parameters evaluated ($P \geq 0.15$; data not shown). However, cows with aggressive temperament had reduced ($P \leq 0.05$) pregnancy and calving rate and tended to have reduced ($P = 0.09$) weaning rate compared with cohorts with adequate temperament (Table 1). In addition, kilogram of calf born per cow exposed to breeding was reduced ($P = 0.05$), whereas kilogram of calf weaned per cow exposed to breeding tended to be reduced ($P = 0.08$) in aggressive cows compared with cohorts with adequate temperament (Table 1). No differences were detected ($P \geq 0.45$) between temperament type for pregnancy loss, calf loss from birth to weaning, calf birth and weaning BW, and weaning age (Table 1). Therefore, differences detected for cow-calf performance variables should be mainly attributed to reduced reproductive ability of aggressive cows. It is also important to note that temperament type × location interactions were not detected ($P \geq 0.32$) for the variables evaluated herein, indicating that temperament impacted cow performance independently of location and its inherent properties, such as breeding procedure and management system.

Supporting the results reported herein, cattle with excitable temperament experience stimulated function of the hypothalamic-pituitary-adrenal axis when exposed to

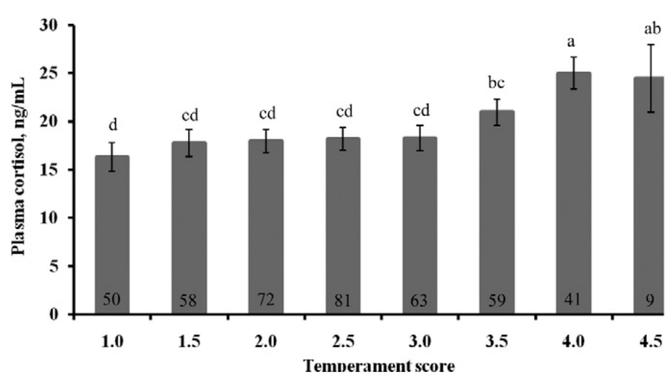

Figure 1. Plasma cortisol concentrations (±SEM) of *Bos taurus* beef cows according to temperament score, which was calculated by averaging cow chute score and exit score. Exit score was calculated by dividing exit velocity results into quintiles and assigning cows with a score from 1 to 5 (exit score: 1 = slowest cows, 5 = fastest cow). A temperament score effect was detected ($P < 0.01$). Values within each bar correspond to number of cows with each temperament score. [a–d] Means with different superscripts differ at $P < 0.05$.

human handling, resulting in a neuroendocrine stress response characterized by increased synthesis and circulating concentrations of ACTH and cortisol (Stahringer et al., 1990; Curley et al., 2008). These stress-related hormones directly impair the physiological mechanisms required for fertility in beef cows, including resumption of estrous cycles, ovulation of a competent oocyte, and establishment of pregnancy (Dobson et al., 2001). More specifically, increased circulating concentrations of ACTH and cortisol disrupt synthesis and release of gonadotropins (Li and Wagner, 1983; Dobson et al., 2000), reduce the sensitivity of the brain to estrogen (Hein and Allrich, 1992), and impair progesterone production by the corpus luteum (Wagner et al., 1972; da Rosa and Wagner, 1981). In the present experiment, reduced pregnancy rates of aggressive cows at the Union location could be attributed, at least partially, to neuroendocrine stress responses stimulated during handling for estrus synchronization and AI (Cooke et al., 2011). However, cows were immediately exposed to bull breeding after AI, which prevents proper differentiation between pregnancies to AI or natural breeding. In addition, cows at the Burns location were exposed only to bull breeding on range pastures with no human interaction or handling to stimulate the neuroendocrine stress responses, whereas no temperament type × location interaction was detected for any of the variables analyzed herein ($P \geq 0.23$). Therefore, additional mechanisms associating temperament and reproduction in beef females, including postconception effects and potential genetic and innate deficiencies within the reproductive system of aggressive cows, warrant further investigation.

In conclusion, results from this experiment indicate that *B. taurus* beef cows with aggressive temperament have impaired reproductive performance and overall productivity compared with cohorts with adequate temperament. Additional research is still required to fully comprehend the effects of temperament on reproductive function of beef females. Nevertheless, management strategies to improve temperament of the cow herd will likely benefit reproductive and consequent production efficiency of cow-calf operations. These may include selection or culling criteria based on cattle temperament or even acclimation to human handling as discussed in Exp. 2. However, previous research from our group demonstrated that acclimation of mature cows to human interaction did not improve temperament and reproductive performance, and such a strategy may not be practical in range cow-calf production systems (Cooke et al., 2009a).

Experiment 2

No treatment effects were detected ($P = 0.37$) for heifer ADG (Table 2). Similarly, no treatment effects were detected for heifer BW change during the study ($P = 0.91$; data not shown). These outcomes were expected given that heifers from both treatments were maintained in similar pastures and provided similar diets. Conversely, in our previous research work with *B. indicus*–influenced cattle (Cooke et al., 2009b), acclimated heifers had reduced ADG compared with control cohorts, which was attributed to altered grazing behavior and additional exercise that acclimated heifers were exposed to during the acclimation period. In the present experiment, heifers had to walk 0.6 km during each acclimation event, whereas acclimated heifers evaluated by Cooke et al. (2009b) had to walk nearly 2 km. Therefore, the shorter walking distance required for the acclimation procedure in the present experiment likely prevented reduced ADG in acclimated heifers.

After the acclimation process, acclimated heifers had reduced ($P = 0.01$) plasma concentrations of cortisol and haptoglobin compared with control cohorts (Table 2). Supporting these results, our and other research groups indicated that acclimation of cattle to handling procedures is an alternative to prevent increased concentrations of cortisol in response to handling stress (Crookshank et al., 1979; Curley et al., 2006; Cooke et al., 2009b). Further, cortisol is known to elicit an inflammatory reaction and increase circulating concentrations of acute-phase proteins (Cooke and Bohnert, 2011), which may explain the reduced plasma haptoglobin concentrations in acclimated heifers compared with control cohorts. No treatment effects were detected ($P \geq 0.69$) for temperament score or chute score (Table 2). However, a treatment × day interaction was detected ($P < 0.01$) for exit velocity because acclimated heifers had similar exit

Table 2. Average daily gain, pregnancy rates, plasma concentrations of cortisol and haptoglobin, and temperament measurements of heifers exposed (n = 44) or not (control; n = 44) to handling acclimation procedures[1]

Item	Acclimated	Control	SEM	*P*-value
ADG,[2] kg/d	0.47	0.46	0.01	0.37
Cortisol,[3] ng/mL	26.1	32.8	1.9	0.01
Haptoglobin,[3] 450 nm × 100	1.04	1.15	0.03	0.01
Chute score	1.89	1.92	0.08	0.79
Temperament score[4]	2.42	2.49	0.12	0.69
Pregnancy rates,[5] %	78.2	86.8	5.3	0.26

[1]Acclimated heifers were exposed to a handling process 3 times weekly for 4 wk (d 11 to 39), which was applied individually to heifers by processing them through a handling facility. Control heifers remained undisturbed on pasture.

[2]Calculated using initial (d 1) and final (d 192) shrunk BW.

[3]Least-squares means adjusted covariately to values obtained before acclimation period (d 10).

[4]Calculated by averaging heifer chute score (Cooke et al., 2011) and exit score. Exit score was calculated by dividing chute exit velocity results into quintiles and assigning heifers with a score from 1 to 5 (exit score; 1 = slowest heifers, 5 = fastest heifers).

[5]Calculated as pregnant heifers/total heifers. Heifers were exposed to breeding procedures beginning on d 205 of the experiment.

velocity on d 0 and 40 ($P \geq 0.13$) but reduced velocity ($P = 0.02$) on d 200 compared with control cohorts (Figure 2). On the basis of our hypothesis, it was expected that temperament score, as well as chute score and exit velocity, would be reduced in acclimated heifers at the end of the acclimation period and support the results detected for plasma concentrations of cortisol and haptoglobin. Conversely, only chute score was altered by the acclimation process in our previous research work with *B. indicus* heifers (Cooke et al., 2009b), although this outcome was cautioned because of the flaws associated with this temperament measurement (Burrow and Corbet, 2000; Cooke et al., 2009a, 2011). Nevertheless, reduced exit velocity at the end of the present experiment indicates that the acclimation process improved, at least partially, behavior of acclimated heifers during handling procedures.

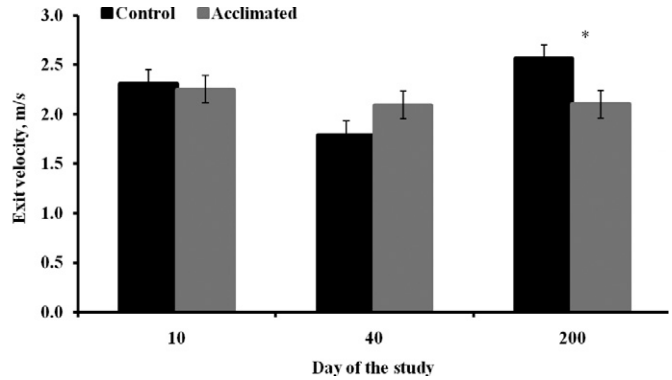

Figure 2. Exit velocity (±SEM) of *Bos taurus* replacement heifers exposed (n = 44) or not (control; n = 44) to handling acclimation procedures. Acclimated heifers were exposed to a handling process 3 times weekly for 4 wk (d 11 to 39), which was applied individually to heifers by processing them through a handling facility, whereas control heifers remained undisturbed on pasture. A treatment × day interaction was detected ($P < 0.01$). Treatment comparison within day: *$P = 0.02$.

A treatment × day interaction was detected ($P = 0.01$) for puberty attainment. Although age at puberty in cattle is highly determined by BW and growth rate (Schillo et al., 1992), acclimated heifers experienced hastened attainment of puberty compared with control heifers (Figure 3) despite their similar ADG (Table 2). Before breeding, a greater ($P < 0.01$; Figure 3) number of acclimated heifers were pubertal compared with control cohorts (59.5% vs. 37.8% pubertal heifers/total heifers; SEM = 5.0). However, no treatment effects were detected ($P = 0.26$) for pregnancy rates (Table 2). Supporting our main hypothesis and previous research (Cooke et al., 2009b), acclimated heifers in the present experiment had reduced cortisol concentrations and hastened onset of puberty compared with control cohorts. The lack of similar treatment effects on pregnancy rates could be attributed, at least partially, to the estrus synchronization protocol used herein given that exogenous GnRH and progesterone may stimulate puberty attainment in heifers, as well as compensate for the detrimental effects of excitable temperament on synthesis and release of steroids and gonadotropins (Patterson et al., 2000; Madgwick et al., 2005; Cooke et al., 2011). Nevertheless, the mechanisms by which acclimation procedures hastened puberty attainment remain unclear. On the basis of our hypothesis, it can be speculated that reduced cortisol concentrations in acclimated heifers facilitated the initiation of the physiological events required for puberty attainment, particularly the first ovulatory LH surge (Li and Wagner, 1983; Dobson et al., 2000). Although concentrations of cortisol were only evaluated when heifers

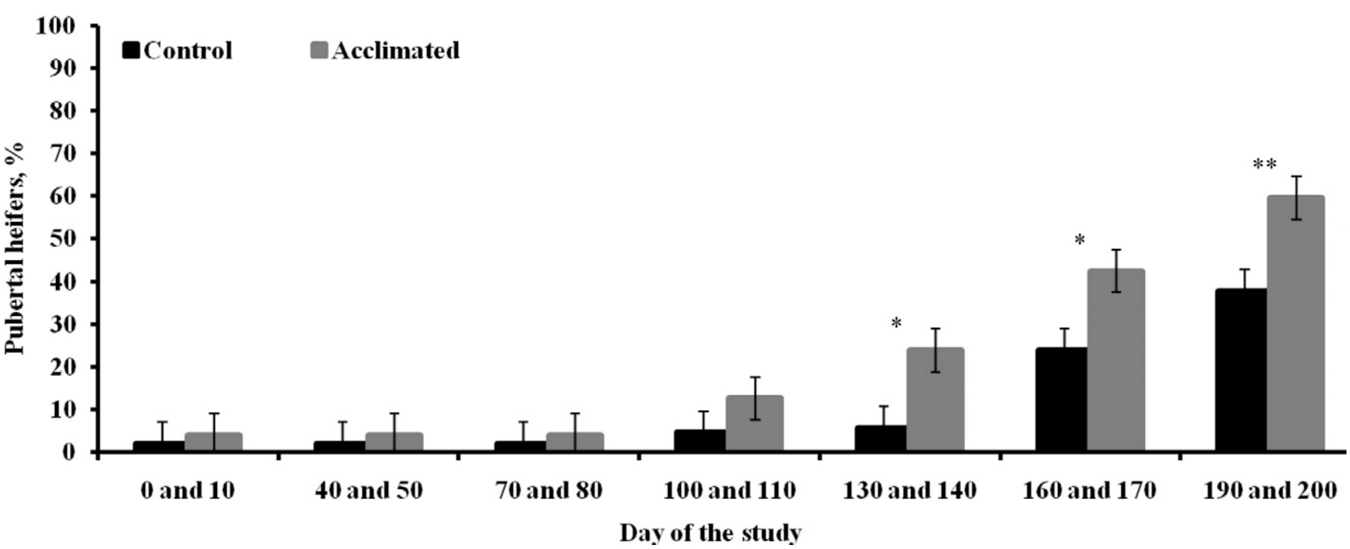

Figure 3. Puberty attainment, according to 10-d interval blood samplings, of heifers exposed (n = 44) or not (control; n = 44) to handling acclimation procedures. Acclimated heifers were exposed to a handling process 3 times weekly for 4 wk (d 11 to 39), which was applied individually to heifers by processing them through a handling facility, whereas control heifers remained undisturbed on pasture. Heifers were considered pubertal when plasma P4 concentrations greater than 1.0 ng/mL were initially and then successively detected within the 10-d interval assessments. A treatment × day interaction was detected ($P = 0.01$). Treatment comparison within days: **$P < 0.01$, *$P = 0.01$.

were handled and restrained for blood collection, it can be speculated that acclimated heifers also had reduced cortisol concentrations compared with control heifers on a daily basis given that both groups were often exposed to brief human interaction, particularly because of feeding and traffic of personnel and vehicles within the research station.

Other mechanisms that may associate acclimation to handling and hastened puberty in beef heifers include the additional exercise that acclimated heifers were exposed to, as well as prepubertal synthesis of P4 by the adrenal gland (Cooke et al., 2009b). More specifically, prepartum exercise regimens enhanced subsequent reproductive efficiency in dairy heifers without impacting BW change (Lamb et al., 1979), whereas exercise stimuli alter circulating concentrations of endogenous opioids that modulate gonadotropin secretion and consequent onset of puberty, cyclicity, and fertility of cattle (Harber and Sutton, 1984; Mahmoud et al., 1989). Progesterone appears to be a required stimulus for puberty establishment in heifers by suppressing the number of estradiol receptors in the hypothalamus and priming the hypothalamic-pituitary-ovarian axis toward enhanced synthesis and pulsatile secretion of LH (Anderson et al., 1996; Looper et al., 2003). In fact, transient increases in circulating concentrations of P4 were reported in beef heifers 2 wk before the onset of puberty (Gonzalez-Padilla et al., 1975). In our previous research with *B. indicus* heifers, a significant proportion of prepubertal heifers experienced increased P4 concentrations during handling (Cooke et al., 2009b), which was attributed to the adrenal gland as this organ synthesizes significant amounts of P4 during a neuroendocrine stress response (Gonzalez-Padilla et al., 1975; Brown, 1994; Cooke and Arthington, 2008). On the basis of this outcome, it was hypothesized that *B. indicus*–influenced acclimated heifers experienced transient increases in P4 synthesis during the acclimation process, particularly during the initial weeks when heifers were still unfamiliar with the acclimation events. However, heifer ovary function was not evaluated in the present experiment, which prevents the assessment of potential contribution of adrenal P4 to puberty attainment. Nevertheless, reduced cortisol concentrations, combined with the additional exercise and potential increases in prepubertal P4, may have all contributed to hastened puberty attainment of acclimated heifers compared with control cohorts.

In conclusion, results from this experiment indicate that acclimation of *B. taurus* heifers to handling procedures and human interaction partially improved heifer temperament, reduced circulating concentrations of stress-related hormones and metabolites, and hastened attainment of puberty. Therefore, acclimation of replacement heifers to human handling after weaning may be an alternative to enhance their reproductive development and increase the efficiency of heifer development programs in cow-calf operations.

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