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Effects of temperament on physiological, productive, and reproductive responses in *Bos indicus* beef cows[1]

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ABSTRACT. This experiment evaluated the effects of temperament on physiological, productive, and reproductive responses in *Bos indicus* beef cows. A total of 953 lactating, multiparous, non-pregnant Nelore cows (age = 99 ± 2 mo; days post-partum = 51.4 ± 0.3 d; BCS = 5.34 ± 0.04; BW = 430 ± 2 kg) were allocated into 8 groups of approximately 120 cows each. Groups were assigned to an estrus synchronization + timed-AI protocol at the beginning of the breeding season. Concurrently with AI, blood samples were collected, hair samples were clipped from the tail switch, and cow temperament was evaluated via chute score and exit velocity. Individual exit score was calculated within each group by dividing exit velocity into quintiles and assigning cows with a score from 1 to 5 (1 = slowest; 5 = fastest cow). Temperament scores were calculated by averaging cow chute score and exit score, and used to define cow temperament (≤ 3 = adequate, *n* = 726; ADQ; > 3 = excitable, *n* = 227; EXC). Cows not pregnant to AI were assigned to a second timed-AI protocol (*n* = 184 ADQ and 72 EXC) or exposed (*n* = 269 ADQ and 90 EXC) to bulls for 60 d. Pregnancy status was verified 30 d after each AI and 45 d after the breeding season via transrectal ultrasound. Cow age, BW, BCS, and d post-partum at the beginning of the breeding season were similar ($P \geq 0.27$) between ADQ and EXC cows. At first timed-AI, EXC had greater ($P < 0.01$) serum cortisol but similar ($P \geq 0.87$) serum haptoglobin and hair cortisol concentrations compared with ADQ cows (49.1 vs. 39.1 ng/mL of serum cortisol, SEM = 1.0). Pregnancy rate to first timed-AI tended ($P = 0.09$) to be less in EXC vs. ADQ cows (41.0 vs. 47.3%; SEM = 3.6), whereas no treatment differences were detected ($P \geq 0.23$) for subsequent pregnancy outcomes. Calving rate was less ($P = 0.04$) in EXC vs. ADQ cows (68.3 vs. 74.8%; SEM = 2.2), which can be attributed to the greater ($P = 0.05$) pregnancy loss detected in EXC cows (9.9 vs. 5.9%; SEM = 1.4). Weaning rate tended ($P = 0.09$) to be less, whereas calf weaning BW and age were less ($P \leq 0.05$) in EXC vs. ADQ cows (63.9 vs. 69.4%, SEM = 2.4; 209 vs. 212 d, SEM = 1; 204 vs. 210 kg, SEM = 2). Hence, kg of calf weaned/cow exposed to breeding was reduced ($P = 0.04$) in EXC vs. ADQ cows (130 vs. 146 kg, SEM = 5). In summary, *B. indicus* cows with excitable temperament had reduced reproductive performance and overall productivity compared to cohorts with adequate temperament when exposed to timed-AI + natural breeding.

Key words: beef cows, *Bos indicus*, production, reproduction, temperament

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INTRODUCTION

Temperament is defined as the fear-related behavioral responses of cattle when exposed to human handling (Fordyce et al., 1988), whereas excitable temperament has been shown to impair reproductive performance in *Bos taurus*-influenced cows (Cooke et al., 2009; Cooke et al., 2012). This outcome was associated with increased circulating cortisol concentrations in cows with excitable temperament (Cooke, 2014), given that cortisol impairs fertility and pregnancy maintenance (Dobson et al., 2001). Moreover, *B. taurus* cows with excitable temperament had reduced calving rate, weaning rate, and kg of calf weaned/cow exposed to breeding compared to cows with adequate temperament, indicating that excitable temperament impacts overall production efficiency in cow-calf systems (Cooke et al., 2012).

Excitable temperament is observed more frequently in *B. indicus* compared with *B. taurus*-influenced cattle (Hearnshaw and Morris, 1984; Fordyce et al., 1988). Given that *B. indicus* breeds are predominant in tropical and subtropical regions of the planet, our research group also investigated and reported reduced pregnancy rates to fixed-time AI in Nelore cows with excitable temperament (Cooke et al., 2011). However, research is still required to further comprehend the impacts of temperament on productive and reproductive outcomes in *B. indicus* cowherds. These include reproductive and overall productivity in females following a typical AI + bull breeding season (Vasconcelos et al., 2014), calving season, and at offspring weaning. In addition, physiological links between temperament and reproductive function need to be evaluated in *B. indicus* cows (Cooke et al., 2011) including cortisol concentrations in hair from the tail switch, which has been recently identified as biomarker of chronic stress in cattle (Moya et al., 2015). Therefore, this experiment investigated the impacts of temperament on physiological, productive, and reproductive parameters of *B. indicus* cows.

MATERIALS AND METHODS

This experiment was conducted from January 2015 to August 2016 in a commercial cow-calf operation located in Nova Xavantina, MT, Brazil. The animals utilized herein were cared for in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Animals and reproductive management

A total of 953 lactating, multiparous, non-pregnant Nelore cows (age = 99 ± 2 mo, d post-partum = 51.4 ± 0.3 d; BW = 430 ± 2 kg, and BCS = 5.34 ± 0.04 according to Wagner et al., 1988) were assigned to the experiment. Cows were allocated into 8 groups of approximately 120 cows each (range = 114 to 123 cows/group) according to the general management scheme of the operation. Groups were maintained in individual *Brachiaria brizantha* pastures with ad libitum access to water and a commercial mineral-vitamin mix (DSM Produtos Nutricionais Brasil, São Paulo, SP, Brazil).

Reproductive management also followed the management scheme of the operation, and was independent of cattle temperament and the objectives of this experiment. All groups were assigned to an estrus synchronization + fixed-time AI protocol at the beginning of the 2015 breeding season (Meneghetti et al., 2009). Within each group, cows were inseminated by 1 of 2 technicians with semen from 2 different Angus sires. Pregnancy status to first timed-AI was verified by detecting a viable conceptus with transrectal ultrasonography (5.0-MHz transducer; 500V, Aloka, Wallingford, CT) 30 d after AI. Cows diagnosed as non-pregnant were either exposed immediately to mature Nelore bulls (1:20 bull to cow ratio) for 60 d (n = 242 cows from 3 groups), assigned to a second synchronization + fixed-time AI protocol (Meneghetti et al., 2009; n = 256 cows from 5 groups), or culled (n = 19). Cows assigned to the second timed-AI were inseminated by 1 of 2 technicians with semen from 2 different Angus sires within each group, and pregnancy status was verified 30 d after AI as previously described. Cows diagnosed as non-pregnant to second timed-AI from 4 of the groups (n = 117) were exposed to mature Nelore bulls (1:20 bull to cow ratio) for 60 d, whereas cows diagnosed as non-pregnant to second timed-AI from 1 of the groups (n = 32) were not exposed to bull breeding and culled from the operation. All bulls utilized in this experiment were submitted to and approved by a breeding soundness evaluation (Chenoweth and Ball, 1980) before the breeding season.

Final pregnancy status was verified by detecting a viable fetus with transrectal ultrasonography (5.0-MHz transducer; 500V, Aloka, Wallingford, CT) 45 d after the end of breeding season. Pregnancy loss was calculated based on pregnancy diagnosis after the breeding season and actual calving rates. Calving was completed within a 15-wk interval for all groups. Calf birth date was recorded and used for calving distribution analysis, which was based on a 15-wk calving season within each group. Calf BW was determined at weaning.

Sampling and temperament evaluation

Cow BW and BCS (Wagner et al., 1988) were recorded at the beginning of the breeding season, when cows were processed for the first time during the estrus synchronization protocol (Meneghetti et al., 2009). Blood samples were collected and hair samples were clipped from the tail switch (Burnett et al., 2014) when cows were restrained for the first timed-AI. Blood samples were collected from either the coccygeal vein or artery into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ). Hair was collected using scissors as close to the skin as possible, and the hair material closest to the skin (2.5 cm of length, 300 mg of weight) was stored at -20°C until processed for cortisol extraction.

Individual cow temperament was assessed by chute score and exit velocity as previously described by Cooke (2014), concurrently with the first timed-AI. Chute score was assessed by a single technician based on 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). Cows were divided within group into quintiles according to their exit velocity, and 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 (**ADQ**; temperament score ≤ 3) or excitable temperament (**EXC**; temperament score > 3).

Laboratorial analyses

Blood samples were placed immediately on ice after collection, allowed to clot for 24 h at 4°C, centrifuged at 1000 × *g* at room temperature for 15 min for serum collection, and stored at -20°C. Serum cortisol concentrations were determined using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Serum haptoglobin concentrations were determined according to the colorimetric procedure described by Cooke and Arthington (2013). The intra- and inter-assay CV were, respectively, 3.0 and 2.1% for serum cortisol, and 2.2 and 5.7% for serum haptoglobin.

Cortisol was extracted from hair samples based on the procedures described by Moya et al. (2013). Briefly, hair samples were cleaned with warm water (37°C) for 30 min, and dried at room temperature for 24 h. Hair samples were then washed twice with isopropanol, dried at room temperature for 120 h, and ground in a 10-mL stainless steel milling cup with a 12-mm stainless steel ball (Retsch Mixer Mill MM400 ball mill; Retsch, Hannover, Germany) for 5 min at a frequency of 30 repetitions/s. Twenty mg of ground hair and 1 mL of methanol were combined into a 7-mL glass scintillation vial, sonicated for 30 min, and incubated for 18 h at 50°C and 100 rpm for steroid extraction. Upon incubation, 0.8 mL of methanol was transferred to a 2-mL microcentrifuge tube and evaporated at 45°C. Samples were reconstituted in 100 µL of the PBS supplied with a salivary cortisol ELISA kit (Salimetrics Expanded Range, High Sensitivity 1-E3002, State College, PA), and stored at -80°C. Samples were analyzed for cortisol concentrations using the aforementioned ELISA kit, whereas intra- and inter-assay CV were, respectively, 1.9 and 3.3%.

Statistical Analyses

All data were analyzed using cow as experimental unit and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. All model statements contained the effect of cow temperament type (ADQ or EXC), whereas model for calving distribution also included the effects of wk and the temperament type × wk interaction. Quantitative data such as cow BCS, serum and hair measurements, and calf weaning BW were analyzed with the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) with cow(temperament type × group) as random variable. Binary data such as pregnancy rates, calving rate and distribution, and pregnancy loss were analyzed with the GLIMMIX procedure of SAS (SAS Inst., Inc) with cow(temperament type × group) as random variable, in addition to sire(group) and AI technician(group) as random variables for pregnancy rates to first and second timed-AI. The probability of cows to become pregnant to first timed-AI was evaluated according to hair cortisol, serum cortisol, and serum haptoglobin concentrations. The GLM procedure of SAS was initially used to determine if each individual measurement influenced pregnancy maintenance linearly, quadratically, or cubically. The LOGISTIC procedure was used to generate the regression model, determine the intercept and slope(s) values according to maximum likelihood estimates from each significant continuous order effect, and the probability of pregnancy was determined according to the following equation: Probability = $(e^{\text{logistic equation}})/(1 + e^{\text{logistic equation}})$. Logistic curves were constructed according to the minimum and maximum values detected for each variable. For all analyses, significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and $P \leq 0.10$.

RESULTS AND DISCUSSION

Based on the temperament evaluation criteria adopted herein, all groups had similar ($P \geq 0.64$) mean temperament score (2.48 ± 0.08) and proportion of EXC

Table 1. Cow and physiological variables in Nelore (*Bos indicus*) beef cows according to temperament

	Temperament type[1]			
Item	Adequate (*n* = 726)	Excitable (*n* = 227)	*SEM*	*P* =
Cow variables[2]				
Age, mo	100	96	2.56	0.27
Days post-partum, d	51.4	51.3	0.43	0.95
BW, kg	431	427	3	0.28
BCS	5.34	5.33	0.05	0.91
Physiological variables[3]				
Serum cortisol, ng/mL	39.1	49.1	1.0	< 0.01
Serum haptoglobin, µg/mL	276	280	21	0.91
Hair cortisol, pg/mg of hair	4.31	4.23	0.23	0.81

[1]Calculated based on cow temperament score (adequate temperament, temperament score ≤ 3; excitable temperament, temperament score > 3) assessed at the first timed-AI of the breeding season (Meneghetti et al., 2009). 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]Values collected (BCS according to Wagner et al., 1988) at the beginning of the breeding season (Meneghetti et al., 2009).

[3]Blood samples and hair samples from the tail switch were collected concurrently with the first timed-AI of the breeding season (Meneghetti et al., 2009).

cows (726 ADQ cows and 227 EXC cows; 23.8% ± 3.9 of EXC cows/total cows). Matsunaga et al. (2002) estimated that the incidence of excitable Nelore cattle in Brazilian beef operations is at 10%, which differs from the results reported herein. This discrepancy can be attributed to several factors, including number of cattle evaluated, differences in cattle population and production systems, as well as temperament evaluation criteria (Cooke et al., 2011). Nevertheless, the goal of this experiment was to investigate the impacts of temperament on reproductive performance and overall productivity in *B. indicus* beef females, and not to determine the incidence of excitable females in *B. indicus* cowherds. The methods used herein to evaluate temperament cattle were similar to our previous research efforts with *B. taurus and B. indicus* cows (Cooke et al., 2011; Cooke et al., 2012; Francisco et al., 2015), and have the purpose of classifying cattle according to temperament characteristics by using techniques that can be feasibly completed during routine cattle processing (Cooke, 2014).

No temperament type effects were ($P \geq 0.27$) detected for cow age and d postpartum at the beginning of the breeding season (Table 1); hence, any physiological, productive and reproductive differences between ADQ and EXC cows should not be related to these variables. In addition, d postpartum values indicate that cows were within the recommended voluntary waiting period for *B. indicus* cattle (Vasconcelos et al., 2014). Cow BCS and BW (Table 1) were also similar ($P \geq 0.28$) between EXC and ADQ cows, which suggests that any effects of temperament type on productive and reproductive outcomes were also independent of cow nutritional status at the beginning of the breeding season (Cooke et al., 2009; Cooke et al., 2011; Cooke et al., 2012).

Serum cortisol concentrations were greater ($P < 0.01$) in EXC vs. ADQ cows at the first timed-AI (Table 1). These results concur with previous findings from our (Cooke et al., 2009; Cooke et al., 2012) and other research groups (Stahringer et al., 1990; Fell et al., 1999; Curley et al., 2006), demonstrating that cattle with excitable temperament have heightened adrenocortical stress reaction during handling compared with calmer cohorts (Burdick et al., 2011; Cooke, 2014). Elevated cortisol has also been positively associated with circulating haptoglobin concentrations in cattle (Cooke et al., 2012), which is a key component of the bovine acute-phase protein response (Carroll and Forsberg, 2007) known to impact cattle productive and reproductive efficiency (Cooke et al., 2009; Araujo et al., 2010). However, no temperament type effects were detected ($P = 0.91$) for serum haptoglobin at the first timed-AI (Table 1), indicating that the increase in adrenocortical activity of EXC cows during handling for timed-AI was not sufficient to concomitantly impact the acute-phase protein reaction. Yet, serum haptoglobin concentrations peak 24 to 72 h after an acute stressor in cattle (Arthington et al., 2008; Cooke and Bohnert, 2011), and serum haptoglobin and cortisol concentrations were analyzed at timed-AI. It is important to note that cows were also handled for estrus synchronization 2, 4, and 11 d prior to timed-AI (Meneghetti et al., 2009). Therefore, similar serum haptoglobin concentration between temperament types at timed-AI also indicates that heightened adrenocortical stress reaction to handling in EXC vs. ADQ cows (Burdick et al., 2011; Cooke, 2014) during estrus synchronization was not sufficient to alter acute-phase protein parameters.

Hair cortisol concentrations were similar ($P = 0.81$) between EXC and ADQ cows (Table 1). Cortisol concentration in hair from the tail switch has been recently identified as biomarker of chronic stress in cattle (Burnett et al., 2014; Marti et al., 2015; Moya et al., 2015), given that cortisol is gradually accumulated in the emerg-

Table 2. Reproductive performance of Nelore (*Bos indicus*) beef cows according to temperament

Item	Temperament type[1]			
	Adequate	Excitable	*SEM*	*P* =
Pregnancy rates,[2,3]%				
First timed-AI	47.3 (341/726)	41.0 (95/227)	3.6	0.09
Second timed-AI	43.1 (79/184)	39.2 (28/72)	5.1	0.56
Natural breeding	58.4 (157/269)	54.4 (49/90)	4.1	0.52
Final (AI + natural)	79.5 (577/726)	75.8 (172/227)	2.1	0.23
Calving rate,[4]%	74.8 (543/726)	68.3 (155/227)	2.2	0.04
Pregnancy loss,[5]%	5.9 (34/577)	9.9 (17/172)	1.4	0.05

[1]Calculated based on cow temperament score (adequate temperament, temperament score ≤ 3; excitable temperament, temperament score > 3) assessed at the first timed-AI of the breeding season (Meneghetti et al., 2009). 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]Cows were assigned to an estrus synchronization + timed-AI protocol at the beginning of the breeding season (Meneghetti et al., 2009). Cows not pregnant to first timed-AI were assigned to a second timed-AI protocol and/or exposed to natural breeding for 50 d. Pregnancy status was verified 30 d after each AI and 45 d after the breeding season via transrectal ultrasonography (5.0-MHz transducer; 500V, Aloka, Wallingford, CT). Values within parenthesis represent pregnant cows divided by cows exposed to AI and/or natural breeding.

[3]Based on the management scheme of the operation, cows not pregnant to first timed-AI (ADQ = 385, EXC = 132) were immediately culled from the operation (ADQ = 14, EXC = 5) or assigned to natural breeding only (ADQ = 187, EXC = 55), second timed-AI only (ADQ = 102, EXC = 37), or second timed-AI followed by natural breeding (ADQ = 82, EXC = 35).

[4]Values within parenthesis represent cows that gave birth to a calf divided by cows exposed to AI and/or natural breeding.

[5]Pregnancy loss was calculated based on pregnancy diagnosis 45 d after the breeding season and calving rates. Values within parenthesis represent cows that lost pregnancy divided by cows diagnosed as pregnant on the end of the breeding season.

ing tail hair and its concentration represents long-term adrenocortical activity (Moya et al., 2013). Research by González-de-la-Vara et al. (2011) suggested that hair cortisol concentrations represent adrenocortical activity during the 14-d interval prior to hair collection, although these authors clipped hair from the coastal region of dairy cattle. Moreover, measuring cortisol in hair from the tail switch eliminates the effects that handling cattle exert on circulating cortisol concentrations (Moya et al., 2013, Moya et al., 2015), given that hair cortisol concentrations are not instantly impacted by the stress of handling (Burnett et al., 2014). In the present experiment, cows were handled 4 times during the 11-d estrus synchronization + protocol (Meneghetti et al., 2009). Hence, the lack of treatment effects on hair cortisol concentrations suggest that increased adrenocortical stress reaction of EXC cows during handling for estrus synchronization + timed-AI was not sufficient to chronically impact adrenocortical activity. Nevertheless, research is still required to explore the associations among temperament, handling frequency, and hair cortisol concentrations in beef cattle.

Pregnancy rate to first timed-AI tended ($P = 0.09$) to be less in EXC vs. ADQ cows (Table 2), corroborating with previous research with *B. indicus* females (Cooke et al., 2011; Rueda et al., 2015). These outcomes can be attributed, at least partially, to the greater adrenocortical stress responses and serum cortisol concentrations (Table 1) in EXC cows stimulated by handling for estrus synchronization and AI. Cortisol directly impairs the physiological mechanisms required for fertility in beef cows (Dobson et al., 2001). These include disrupted synthesis and release of gonadotropins (Li and Wagner, 1983; Dobson et al., 2000), reduced sensitivity of the brain to estrogen (Hein and Allrich, 1992), and impaired progesterone production by the corpus luteum (Wagner et al., 1972; da Rosa and Wagner, 1981). Supporting this rationale, the probability of cows becoming pregnant to first timed-AI was decreased linearly ($P < 0.01$) as serum cortisol concentrations increased (Fig. 1). A similar out-

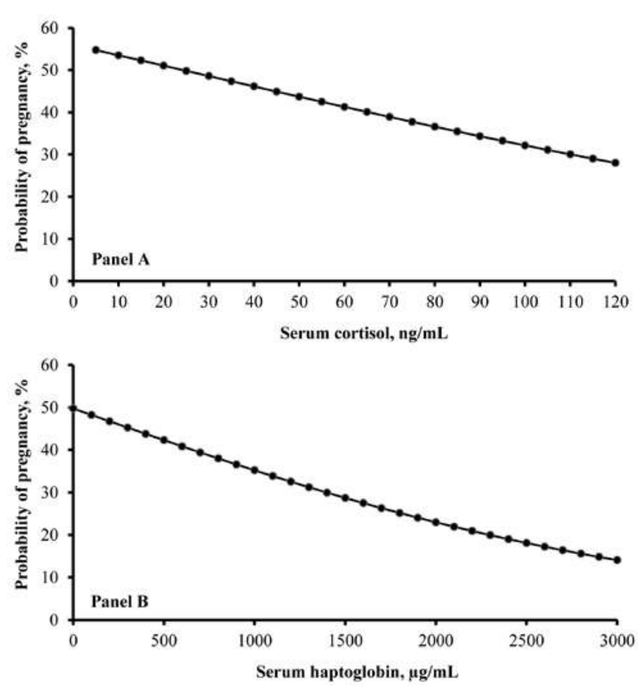

Figure 1. Probability of pregnancy to fixed-time AI in Nelore (*Bos indicus*) beef cows according serum cortisol (panel A) and haptoglobin (panel B) concentrations at the time of AI. Pregnancy status was verified 30 d after AI via transrectal ultrasonography (5.0-MHz transducer; 500V, Aloka, Wallingford, CT). A linear effect was detected ($P < 0.01$) for both variables.

#907

Table 3. Weaning outcomes from Nelore (*Bos indicus*) beef cows according to temperament

	Temperament type[1,2]			
Item	Adequate	Excitable	*SEM*	*P* =
Calf parameters[3]				
Calf weaning age, d	212	209	1	0.05
Calf weaning BW, kg	210	204	2	0.04
Proportion of weaned male calves, %	51.4 (259/504)	52.4 (76/145)	3.2	0.83
Proportion of weaned AI-sired calves, %	74.2 (374/504)	71.7 (104/145)	2.7	0.55
Cow-calf production parameters[4]				
Calf loss from birth to weaning,[4]%	7.2 (39/543)	6.4 (10/155)	1.5	0.75
Weaning rate,[5]%	69.4 (504/726)	63.9 (145/227)	2.4	0.09
Kg of calf weaned per cow exposed,[6] kg	146	130	5	0.04

[1]Calculated based on cow temperament score (adequate temperament, temperament score ≤ 3, *n* = 726; excitable temperament, temperament score > 3, *n* = 227) assessed at the first timed-AI of the breeding season (Meneghetti et al., 2009). Temperament score was calculated by averaging 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]Cows were assigned to an estrus synchronization + timed-AI protocol at the beginning of the breeding season (Meneghetti et al., 2009). Cows not pregnant to first timed-AI were assigned to a second timed-AI protocol and/or exposed to natural breeding for 50 d.

[3]Values within parenthesis represent male or AI-sired calves divided by total weaned calves.

[4]Calf loss was calculated based on calving rate and weaning rate. Values within parenthesis represent number of dead calves divided by total calves born.

[5]Values within parenthesis represent number of calves weaned divided by number of dams exposed to breeding.

[6]Kilograms of calf weaned per cow exposed were calculated based on weaning rate and calf BW at weaning.

come was detected for serum haptoglobin (linear effect, *P* < 0.01; Fig. 1), despite the lack of temperament type effects on this variable. Inflammatory and acute-phase protein responses are known to impair cattle reproductive function by disturbing follicle development, ovulation, and pregnancy establishment (Battaglia et al., 2000; Williams et al., 2001; Hansen et al., 2004). Conversely, hair cortisol concentration was not associated (*P* ≥ 0.21) with pregnancy probability to first timed-AI (data not shown), suggesting that adrenocortical activity prior to and during the estrus synchronization protocol may not impact cow reproductive efficiency to fixed-time AI.

No differences were detected (*P* = 0.23) for pregnancy rates to second timed-AI, natural breeding, and final pregnancy rates (Table 2). The reason for inconsistent results among first timed-AI and subsequent breeding procedures are unknown and cannot be properly addressed herein, particularly because cattle temperament and serum cortisol concentrations were not assessed during these latter events. However, calving rate was less (*P* = 0.04) in EXC vs. ADQ cows (Table 2), which can be attributed to the greater (*P* = 0.05) pregnancy loss detected in EXC vs. ADQ cows (Table 2). Although heightened adrenocortical stress reaction during handling is also expected to impair pregnancy maintenance in cattle with excitable temperament (Merrill et al., 2007; Cooke, 2014), the pregnancy losses observed herein occurred after the end of the breeding season when cattle were seldom handled. Hence, additional mechanisms associating temperament and reproduction in beef females warrant further investigation, including post-conception effects, pregnancy development and maintenance, as well as potential genetic and innate deficiencies within the reproductive system of excitable cows (Cooke, 2014). Accordingly, several genes that may be responsible for cattle temperament have been identified (Schmutz et al., 2001), whereas genetic correlations between temperament and reproductive traits were already reported in Nelore cattle (Barrozo et al., 2012).

No temperament type effects were detected (*P* = 0.75) for calf loss from birth to weaning. Hence, weaning rate tended to be less (*P* = 0.09) in EXC vs. ADQ cows (Table 3), corroborating with results from *B. taurus* cows (Cooke et al., 2012). Weaning BW was greater (*P* = 0.04) in calves from ADQ cows compared with cohorts from EXC cows (Table 3), which can be directly attributed to a similar temperament type effect (*P* = 0.05) detected for calf weaning age (Table 3). The greater weaning age of calves from ADQ cows can be related to temperament type effects detected for first timed-AI and numerical increase in pregnancy rates to second-time AI in ADQ vs. EXC cows (Table 2), which resulted in ADQ cows calving earlier in the breeding season compared with EXC cows (temperament × type interaction, *P* = 0.02; Fig. 2). Conversely, differences in calf weaning BW should not be associated with proportion of male or proportion of AI-sired calves (Marques et al., 2016), as these variables were similar (*P* ≥ 0.55) between EXC and ADQ cows (Table 3). Accordingly, calf weaning BW was similar (*P* = 0.22) between ADQ and EXC when calf weaning age was included as independent covariate (209 vs. 206 kg, respectively; SEM = 2), but was still greater (*P* ≤ 0.05) in ADQ when proportion of male calves weaned (210 vs. 204 kg, respectively; SEM = 2) or AI-

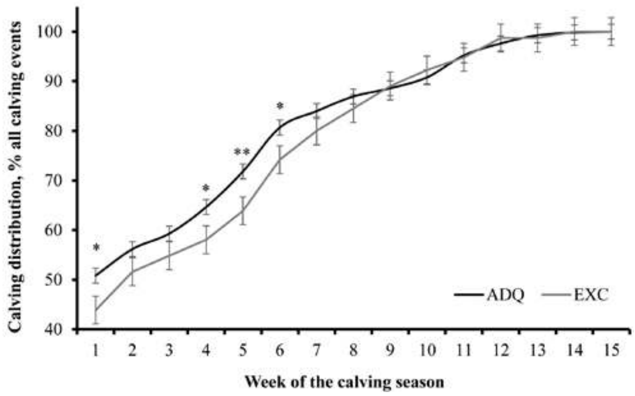

Figure 2. Calving distribution during a 15-wk calving season in Nelore (*Bos indicus*) beef cows classified with excitable (**EXC**) or adequate (**ADQ**) temperament (Cooke, 2014) at the beginning of the previous breeding season. A temperament type × week interaction was detected (P = 0.02). Temperament type comparison within week: ** $P = 0.01$, * $P \leq 0.05$.

sired calves weaned (210 vs. 205 kg, respectively; SEM = 2) were included. Lastly, kg of calf weaned/cow exposed to breeding was less (P = 0.04) in EXC vs. ADQ cows (Table 3), agreeing with results from Cooke et al. (2012) in *B. taurus* females. However, Cooke et al. (2012) did not report temperament type effects on calf weaning BW, likely due to the similar weaning age in calves born from *B. taurus* classified as excitable or adequate temperament.

Collectively, this experiment provides novel information regarding the negative impacts of excitable temperament on reproductive efficiency and overall productivity in *B. indicus* beef females. More specifically, EXC cows experienced reduced pregnancy rates to first timed-AI, increased pregnancy loss, which resulted in decreased calving and weaning rates compared with cows with ADQ cohorts. Moreover, EXC calved later during the calving season, weaned younger and lighter offspring, which resulted in a 16-kg decrease in kg of calf weaned/cow exposed to breeding compared with ADQ cows. The exact biological reason for these outcomes are unknown and warrant investigation, given that serum cortisol results can only help explaining temperament type effects on pregnancy rates to first timed-AI. These include, but are not limited to, physiological and genetic relationships between excitable temperament and reproductive function. Nevertheless, this experiment demonstrated that cattle temperament has direct implications on overall production efficiency in cow-calf system based on *B. indicus* females.

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