

Impacts of meloxicam administration before temporary calf weaning on physiological and reproductive responses of *Bos indicus* beef cows¹

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ABSTRACT: The objective of this experiment was to evaluate temperament, physiological, and reproductive variables in *Bos indicus* beef cows assigned to an estrus synchronization + timed AI protocol including eCG administration, 48-h temporary calf weaning (TCW), or TCW + meloxicam administration. A total of 943 lactating, multiparous, nonpregnant Nelore cows, allocated into 8 groups of approximately 120 cows each, were assigned to the experiment. Groups were maintained in individual pastures and assigned to the following estrus synchronization + timed AI protocol: a 2-mg injection of estradiol benzoate and an intravaginal progesterone-releasing device (CIDR) on d 0, a 12.5-mg injection of PGF_{2α} on d 7, CIDR removal in addition to a 0.6-mg injection of estradiol cypionate on d 9, and timed AI on d 11. Within each group, cows were randomly assigned on d 9 to 1) TCW from d 9 to 11 (TCW-CON; *n* = 317), 2) no TCW and a 300-IU injection of eCG on d 9 (NOTCW; *n* = 311), and 3) TCW-CON in addition to meloxicam administration (intramuscular; 0.5 mg/kg BW) on d 9 (TCW-MEL; *n* = 315). Cow BW and BCS were assessed on d 0. On d 9 and 11, blood samples were collected, and cow temperament was evaluated via chute score and exit velocity. Pregnancy status was

verified 30 d after timed AI via transrectal ultrasonography. No treatment differences were detected ($P \geq 0.23$) for cow age, days postpartum, BW, and BCS on d 0 of the estrus synchronization + timed AI protocol. No treatment effects were detected ($P \geq 0.41$) for any of the temperament variables evaluated. A treatment \times day interaction was detected ($P = 0.02$) for serum cortisol concentrations, which were similar ($P = 0.55$) between treatments on d 9 but greater ($P \leq 0.05$) in TCW-CON and TCW-MEL compared with NOTCW cows on d 11. No treatment effects were detected ($P = 0.90$) for serum haptoglobin concentrations, which decreased from d 9 to 11 in all treatments (day effect; $P < 0.01$). No treatment differences were detected ($P = 0.84$) for pregnancy rates to timed AI. In summary, TCW during estrus synchronization did not impact temperament or serum haptoglobin concentrations in *B. indicus* beef cows but increased serum cortisol concentrations compared with cows not assigned to TCW, although such an outcome was not sufficient to impact pregnancy rates to timed AI. Moreover, administration of meloxicam did not alleviate the TCW-induced increase in serum cortisol concentrations and failed to benefit pregnancy rates to timed AI in *B. indicus* beef cows.

Key Words: artificial insemination, beef cows, meloxicam, reproduction, temporary calf weaning

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INTRODUCTION

Weaning stimulates stress-related temperament and physiological changes in calves and cows (Whisnant et al., 1985; von Keyserlingk and Weary, 2007), such as inflammatory and acute-phase protein responses (Arthington et al., 2005), known to impair reproductive performance in beef females (Cooke et

al., 2009). However, inclusion of temporary calf weaning (48 h; **TCW**) in estrus synchronization protocols based on estradiol + progesterone increased pregnancy rates to timed AI in *Bos indicus* beef cows (Sá Filho et al., 2009), given that TCW promotes adequate LH secretion for follicular growth and ovulation (Williams et al., 1983; Edwards, 1985). One alternative to prevent the stress associated with TCW during estrus synchronization but still enhance preovulatory follicular development is to replace TCW with a single eCG administration (Kuran et al., 1996). Nevertheless, Sá Filho et al. (2009) reported similar pregnancy rates to timed AI in *B. indicus* cows assigned to TCW, eCG administration, or both. Therefore, strategies that alleviate stress-induced responses in *B. indicus* beef cows receiving TCW may further improve pregnancy rates to timed AI compared with conventional TCW or eCG treatment.

Administration of meloxicam, a nonsteroidal anti-inflammatory drug, to cattle exposed to stressful procedures alleviated the resultant inflammatory reactions (Guarnieri Filho et al., 2014; Van Engen et al., 2014). Moreover, meloxicam has a half-life of 28 h and remains in the circulation for 5 d after a single administration to cattle (Van Engen et al., 2014). On the basis of this rationale, we hypothesized that meloxicam administration to beef cows mitigates TCW-induced inflammatory reactions and further improves pregnancy rates to timed AI. Therefore, this experiment evaluated temperament, physiological, and reproductive variables in *B. indicus* beef cows assigned to an estrus synchronization + timed AI protocol including eCG administration, TCW, or TCW + meloxicam administration.

MATERIALS AND METHODS

This experiment was conducted from January to February 2015 on a commercial cow-calf operation located in Nova Xavantina, 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 (Federation of Animal Science Societies, 2010).

Animals and Treatments

A total of 943 lactating, multiparous, nonpregnant Nelore cows (age = 99 ± 2 mo, days postpartum = 51.4 ± 0.3 d; BW = 430 ± 2 kg, and BCS = 5.34 ± 0.03 , according to Wagner et al. [1988] and assessed by a single technician) were assigned to the experiment. After calving, 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, and this arrangement was maintained throughout the

experimental period. Groups were maintained in individual *Brachiaria brizantha* pastures (average stocking rate = 1 cow-calf pair/ha) with ad libitum access to water and a commercial mineral-vitamin mix (DSM Produtos Nutricionais Brasil, São Paulo, Brazil). All groups were assigned to the same estrus synchronization + timed AI protocol; Meneghetti et al., 2009; d 0 to 11). More specifically, cows received a 2-mg injection (intramuscular [**i.m.**]) of estradiol benzoate (Gonadiol; Zoetis, São Paulo, Brazil) and an intravaginal progesterone releasing device (**CIDR**, originally containing 1.9 g of progesterone; Zoetis) on d 0, a 12.5-mg injection (i.m.) of PGF_{2α} (Lutalyse; Zoetis) on d 7, CIDR removal in addition to a 0.6-mg injection (i.m.) of estradiol cypionate (**ECP**; Zoetis) on d 9, and timed AI on d 11. Within each group, cows were randomly assigned on d 9 of the estrus synchronization + timed AI protocol to 1 of 3 treatments: 1) 48-h TCW from d 9 (immediately after CIDR removal and ECP injection) until after timed AI on d 11 (**TCW-CON**; $n = 317$), 2) no TCW and a 300-IU injection (i.m.) of eCG (Novormon, Zoetis) administered concurrently with ECP on d 9 (**NOTCW**; $n = 311$), and 3) the same as TCW-CON, with the addition of meloxicam administration (11-mL i.m. injection of Maxicam 2%; Ourofino Saúde Animal, Cravinhos, Brazil) concurrently with ECP on d 9 (**TCW-MEL**; $n = 315$).

Within each group, cows were inseminated by 1 of 2 technicians with semen from 2 different sires. The proportion of cows inseminated by each technician, sire, and the combination of technician and sire were balanced within each group. From d 9 to d 11, TCW-CON and TCW-MEL cows returned to pasture and had no visual contact with their calves, which remained inside the working facility with ad libitum access to water and a pelletized concentrate (DSM Produtos Nutricionais Brasil), while NOTCW cows and their calves were maintained in the same pasture as TCW-CON and TCW-MEL cows. After timed AI on d 11, TCW-CON and TCW-MEL cows were reunited with their respective calves, and all cow-calf pairs returned to pasture. The meloxicam dose utilized herein was based on the average BW of the herd on d 0 (430 ± 2 kg) to result in 0.5 mg of meloxicam administered per kilogram of cow BW, which is the injectable dose suggested by the manufacturer and used in previous research with dairy cows (Amiridis et al., 2009; Erdem and Guzeloglu, 2010).

Sampling

Cow BW and BCS were assessed on d 0 of the estrus synchronization + timed AI protocol. Cow temperament was evaluated, and blood samples were collected immediately before treatment application on d 9 and immediately before timed AI on d 11. Pregnancy

status was verified 30 d after timed AI by detecting a viable conceptus with transrectal ultrasonography (5.0-MHz transducer; 500V, Aloka, Wallingford, CT). Cows were not exposed to bulls or to additional AI services between timed AI on d 11 and pregnancy evaluation.

Cow temperament was assessed by chute score and exit velocity as previously described by Cooke et al. (2011a) by a single technician. Chute score was assessed 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 by determining the speed of the cow exiting the squeeze chute by measuring rate of travel over a 1.8-m distance with an infrared sensor (FarmTek Inc., North Wylie, TX). In addition, cows were divided in quintiles within each group 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.

Blood samples were collected from either the coccygeal vein or artery into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ). After collection, blood samples were placed immediately on ice, allowed to clot for 24 h at 4°C, centrifuged at 3,000 × 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 interassay CV were, respectively, 3.0% and 2.1% for cortisol and 2.2% and 7.8% for haptoglobin.

Statistical Analysis

Quantitative (cow age, BCS, BW, days postpartum, serum variables, and temperament variables) and binary (pregnancy outcomes) data were analyzed, respectively, with the MIXED and GLIMMIX procedures of SAS (SAS Inst. Inc., Cary, NC; version 9.3) and the Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects, using cow as the experimental unit and cow(treatment × group) as the random variable. The model statement used for analysis of cow age, days postpartum, BW, and BCS on d 0 contained the effects of treatment, group, and the resultant interaction. The model statement used for analysis of temperament and serum variables contained the effects of treatment, day, group, and all resultant in-

Table 1. Age, days postpartum, BW, and BCS of *B. indicus* beef cows assigned to an estrus synchronization protocol including temporary calf weaning (TCW-CON; *n* = 317), TCW-CON + meloxicam administration (TCW-MEL; *n* = 315), or no temporary calf weaning (NOTCW; *n* = 311)¹

Item ²	NOTCW	TCW-CON	TCW-MEL	SEM	<i>P</i> -value
Age, mo	101.7	97.4	98.5	2.8	0.52
Days postpartum	51.3	51.8	50.9	0.5	0.40
BW, kg	430	427	433	3	0.23
BCS	5.34	5.27	5.39	0.06	0.38

¹Cows were assigned to an estrus synchronization + timed AI protocol (Meneghetti et al., 2009) from d 0 to 11. On d 9, cows were randomly assigned to 1) TCW-CON = 48-h TCW from d 9 until after timed AI on d 11, 2) NOTCW = no TCW and a 300-IU injection (intramuscular [i.m.] of eCG (Novormon, Zoetis, São Paulo, Brazil) administered on d 9, and 3) TCW-MEL = TCW + meloxicam administration (11-mL i.m. injection of Maxicam 2%, Ourofino Saúde Animal, Cravinhos, Brazil) on d 9.

²Cow age, days postpartum, BW, and BCS (Wagner et al., 1988; assessed by a single technician) were recorded on d 0.

teractions. The specified term for the repeated statement was day, cow(treatment × group) was the subject, and the covariance structure utilized for all repeated statements was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. The model statement used for analysis of pregnancy rates to timed AI contained the effects of treatment, group, and the resultant interaction in addition to sire(group) and AI technician(group) as random variables. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10 . Results were separated using PDIFF and are reported as least squares means according to the main treatment effect if no interactions were significant or according to the highest-order interaction detected that contained the effect of treatment.

RESULTS AND DISCUSSION

No treatment differences were detected ($P \geq 0.23$) for cow age, days postpartum, BW, and BCS on d 0 of the estrus synchronization + timed AI protocol (Table 1), indicating that any treatment effects reported herein were independent of these variables. Further, all cows utilized herein were in adequate nutritional status according to their BCS and within the recommended voluntary waiting period for *B. indicus*-influenced cattle to optimize pregnancy rates to timed AI (Cooke et al., 2009) and maintain a 365-d calving interval (Vasconcelos et al., 2014).

No treatment effects were detected ($P \geq 0.41$) for any of the temperament variables evaluated (Table 2). The process of separating cows from their calves, whether temporary or permanent, is known to impact cow behavioral traits, including increased vocaliza-

Table 2. Temperament measurements of *B. indicus* beef cows assigned to an estrus synchronization protocol including temporary calf weaning (TCW-CON; $n = 317$), TCW-CON + meloxicam administration (TCW-MEL; $n = 315$), or no temporary calf weaning (NOTCW; $n = 311$)¹

Item ²	NOTCW	TCW-CON	TCW-MEL	SEM	P-value
Chute score					
d 9	1.94	1.92	1.93	0.04	0.82
d 11	1.96	1.95	1.95	0.04	0.98
Exit velocity, m/s					
d 9	2.11	2.14	2.17	0.05	0.73
d 11	2.23	2.32	2.24	0.05	0.41
Exit score					
d 9	2.98	3.00	3.04	0.08	0.86
d 11	3.00	3.00	3.02	0.08	0.98
Temperament score					
d 9	2.46	2.46	2.50	0.05	0.85
d 11	2.48	2.48	2.48	0.05	0.99

¹Cows were assigned to an estrus synchronization + timed AI protocol (Meneghetti et al., 2009) from d 0 to 11. On d 9, cows were randomly assigned to 1) TCW-CON = 48-h TCW from d 9 until after timed AI on d 11, 2) NOTCW = no TCW and a 300-IU injection (intramuscular [i.m.]) of eCG (Novormon, Zoetis, São Paulo, Brazil) administered on d 9, and 3) TCW-MEL = TCW + meloxicam administration (11-mL i.m. injection of Moxicam 2%, Ourofino Saúde Animal, Cravinhos, Brazil) on d 9.

²Temperament was assessed as described by Cooke et al. (2011a).

tion and physical activity (von Keyserlingk and Weary, 2007). Nevertheless, the impacts of TCW on cow temperament during the estrus synchronization protocol still warranted investigation, given that temperament has been shown to influence productive and reproductive performance in cattle (Cooke et al., 2009, 2011a, 2012a). Using this rationale, we theorized that TCW would increase cow excitement during handling as evidence that TCW causes stress-induced temperament changes in *B. indicus* beef cows. However, the lack of treatment effects on cow temperament reported herein indicates that the TCW process was not sufficient to alter cow temperament during handling from d 9 to 11.

A treatment \times day interaction was detected ($P = 0.02$) for serum cortisol. On d 9, serum cortisol concentrations were similar ($P = 0.55$) between treatments; however, serum cortisol concentrations were greater ($P \leq 0.05$) in TCW-CON and TCW-MEL cows compared with NOTCW cohorts on d 11 (Table 3). In addition, serum cortisol concentrations increased from d 9 to 11 in TCW-CON and TCW-MEL cows (day effect, $P < 0.01$) but not in NOTCW cows (day effect, $P = 0.22$; Table 3). These results corroborate our hypothesis and previous research (Whisnant et al., 1985) reporting that TCW elicits a neuroendocrine stress response in beef cows. However, meloxicam administration did not prevent or alleviate the TCW-induced cortisol response, given that serum corti-

Table 3. Serum concentrations of cortisol and haptoglobin and pregnancy rates in *B. indicus* beef cows assigned to an estrus synchronization protocol including temporary calf weaning (TCW-CON; $n = 317$), TCW-CON + meloxicam administration (TCW-MEL; $n = 315$), or no temporary calf weaning (NOTCW; $n = 311$)¹

Item	NOTCW	TCW-CON	TCW-MEL	SEM	P-value
Cortisol, ng/mL					
d 9	37.8	38.2	39.3	1.1	0.55
d 11	38.9 ^a	41.8 ^b	43.9 ^b	1.1	<0.01
Haptoglobin, ng/mL					
d 9	0.354	0.355	0.324	0.027	0.66
d 11	0.254	0.281	0.296	0.027	0.54
Pregnancy rates, ² %					
	46.6 (145/311)	45.8 (145/317)	44.3 (139/315)	3.6	0.84

^{a,b}Within rows, values with different letters differ ($P < 0.05$).

¹Cows were assigned to an estrus synchronization + timed AI protocol (Meneghetti et al., 2009) from d 0 to 11. On d 9, cows were randomly assigned to 1) TCW-CON = 48-h TCW from d 9 until after timed AI on d 11, 2) NOTCW = no TCW and a 300-IU injection (intramuscular [i.m.]) of eCG (Novormon, Zoetis, São Paulo, Brazil) administered on d 9, and 3) TCW-MEL = TCW + meloxicam administration (11-mL i.m. injection of Moxicam 2%, Ourofino Saúde Animal, Cravinhos, Brazil) on d 9.

²Pregnancy status to timed AI was verified by detecting a viable conceptus with transrectal ultrasonography (5.0-MHz transducer; 500V, Aloka, Wallingford, CT) on d 41. Values reported within parentheses correspond to number of pregnant cows/total cows assigned to timed AI.

sol concentrations between TCW-CON and TCW-MEL cows were similar ($P = 0.24$) on d 11. Guarnieri Filho et al. (2014) and Van Engen et al. (2014) also reported that meloxicam administration to transported feeder cattle did not impact circulating cortisol concentrations after transport and feedlot entry. Accordingly, meloxicam is known to inhibit cyclooxygenase-2 activity and subsequent production of PG and other inflammatory compounds (Griswold and Adams, 1996), whereas its impacts on adrenal corticoid synthesis, if any, still require investigation (Van Engen et al., 2014).

No treatment effects were detected ($P = 0.90$) for serum haptoglobin concentrations (Table 3). The stress caused by cow and calf separation elicits inflammatory responses including the acute-phase protein reaction, which results in nonspecific increases in plasma concentrations of acute-phase proteins such as haptoglobin (Arthington et al., 2005). In fact, the acute-phase protein reaction can be activated by several weaning stressors, including altered temperament (Francisco et al., 2012), elevated cortisol concentrations (Cooke and Bohnert, 2011; Cooke et al., 2012b), and potential decreases in cow DM and water intake during TCW (von Keyserlingk and Weary, 2007; Marques et al., 2012). Nevertheless, the vast majority of research evaluating the impacts of weaning on inflammatory and acute-phase protein reactions focused on the calf (Arthington et al., 2005, 2008; Cooke et al., 2011b), and little is known about its impacts

on the cow. In the present experiment, the lack of treatment effects and the fact that serum haptoglobin concentrations decreased from d 9 to 11 in all treatments (day effect, $P < 0.01$; Table 3) suggest that TCW did not stimulate an acute-phase protein reaction. Moreover, the lack of TCW-induced acute-phase protein reaction rendered meloxicam administration unnecessary, which may explain the similar serum haptoglobin concentrations between TCW-CON and TCW-MEL cows. Perhaps the stress-related responses elicited by TCW, such as serum cortisol, were not sufficient to stimulate an acute-phase protein reaction. One can also speculate that the sampling schedule adopted herein was not adequate to properly assess TCW-induced serum haptoglobin responses. However, circulating haptoglobin concentrations are increased from 24 to 72 h in beef calves exposed to the stress of weaning and road transport (Arthington et al., 2008; Araujo et al., 2010; Francisco et al., 2012). To our knowledge, this is the first research effort that evaluated serum haptoglobin concentrations in *B. indicus* cows on TCW; therefore, additional research is warranted to further understand this subject.

No treatment differences were detected ($P = 0.84$) for pregnancy rates to timed AI (Table 3). The rationale for this experiment was 1) TCW would increase cow excitement and serum cortisol concentrations (Whisnant et al., 1985; von Keyserlingk and Weary, 2007) during the estrus synchronization process, which in turn would stimulate an acute-phase protein reaction known to impair reproductive performance of *B. indicus* beef cows (Arthington et al., 2005; Cooke et al., 2009), and 2) meloxicam administration at the beginning of TCW would alleviate this latter outcome (Guarnieri Filho et al., 2014; Van Engen et al., 2014) and further improve pregnancy rates to timed AI compared with conventional TCW or eCG administration. This rationale was based on the premise that elevated cortisol and inflammatory compounds are known to directly impair mechanisms associated with fertility in cattle, such as synthesis and activity of GnRH, gonadotropins, and estradiol (Peter et al., 1989; Dobson et al., 2000; Williams et al., 2001). Accordingly, Cooke et al. (2009) reported a negative association among plasma cortisol and haptoglobin concentrations at the beginning of a 90-d breeding season with the probability of *B. indicus*-influenced cows to become pregnant. In the present experiment, our hypothesis was partially accepted because TCW-CON and TCW-MEL cows had greater cortisol concentrations on d 11 compared with NOTCW cows, despite the lack of treatment effects on pregnancy rates to timed AI. Perhaps the magnitude of cortisol differences among TCW-CON and TCW-MEL vs. NOTCW cows on d 11 was not only insufficient to increase serum haptoglobin concentrations in TCW-receiving cows as previously mentioned but

also insufficient to yield differences in pregnancy rates to timed AI. Nevertheless, the similar pregnancy rates to timed AI among treatments support the results reported by Sá Filho et al. (2009) and summarized by Vasconcelos et al. (2014); estrus synchronization + timed AI protocols based on progesterone and estradiol can be associated with either TCW or eCG to enhance reproductive performance of *B. indicus* cows.

In summary, results from this experiment indicate that inclusion of TCW into an estrus synchronization protocol based on estradiol and progesterone did not impact temperament or serum haptoglobin concentrations in *B. indicus* beef cows but increased serum cortisol concentrations compared with cohorts not assigned to TCW, although this outcome was not sufficient to impact pregnancy rates to timed AI. In addition, administration of meloxicam did not alleviate the TCW-induced increase in serum cortisol concentrations and failed to improve pregnancy rates to timed AI in *B. indicus* beef cows.

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