



Effects of separation time on behavioral and physiological characteristics of Brahman cows and their calves



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ARTICLE INFO

Article history:

Received 6 August 2015

Received in revised form 9 March 2016

Accepted 17 March 2016

Available online 19 March 2016

Keywords:

Calf

Weaning

Cattle

Welfare

Zebu

Bos indicus

ABSTRACT

The aim of this study was to investigate the effects of separation time on behavioral and physiological characteristics of Brahman cows and their calves. Thirty Brahman cow-calf pairs raised under extensive conditions were randomly assigned to one of three temporary weaning duration: (n = 10/group): (1) 24 h, (2) 48 h, (3) 72 h. Treatment was applied at 25 and 45 days postpartum (dpp), with the same cow-calf pairs allocated to the same weaning duration treatment at both ages. Behaviors recorded include the proximity of cows and calves to the fence separating them, and animals vocalizing and/or lying down. Serum cortisol concentration in cows and calves, and calves weight loss and milk intake were calculated as physiological and productivity measures. At 25 dpp the percentage of cows at <10 m from the fence-line separating them from their calf, decreased as increasing duration of the separation period increased ($Z = 6.2, p < 0.05$). At 45 dpp this pattern was not observed. The number of cows vocalizing decreased as the separation period increased (37–10% and 13–0%, from 24 to 72 h at 25 and 45 dpp, respectively ($Z = 6.6, p < 0.05$)). More calves vocalized ($Z = 3.6, p < 0.05$) at 24 and 48 h in comparison to 72 h, regardless of their age. In cows, increases in cortisol with respect to pre-treatment concentration were only significant ($F_{2/57} = 6.6, p < 0.05$) when subjected to 72 h separation at 25 dpp, while in calves, increases in cortisol were inversely proportional to the duration of cow-calf separation ($10.3 \pm 2.9, 5.3 \pm 2.2$ and 2.0 ± 1.1 ($F_{2/57} = 6.6, p < 0.05$) vs. $6.2 \pm 2.8, 2.1 \pm 1.4$ and 0.8 ± 0.7 ng/ml ($F_{2/57} = 4.6, p < 0.05$), at 24, 48 and 72 h of separation of 25 vs. 45 day old calves, respectively). A similar relation was observed in weight losses ($5.4 \pm 1.3, 2.7 \pm 0.9$ and 0.2 ± 0.1 ($t = 8.13, p < 0.05$) vs. $2.2 \pm 0.9, 0.6 \pm 0.5$ and 0.7 ± 0.6 kg ($t = 4.15, p < 0.05$), at 24, 48 and 72 h of separation for 25 vs. 45 day old calves, respectively). In conclusion, most stress indicators in cows and calves decreased after 48 h of separation, cows were less affected than their young, and older calves displayed less signs of stress than younger animals.

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1. Introduction

Temporary weaning is a procedure known to reduce the length of anestrus postpartum in beef cows (Das et al., 1999; Galina et al., 2001). It is also well known that the presence of the calf and the suckling stimulus inhibit the onset of ovarian activity (Hoffman et al., 1996). Calf separation between 60 and 90 days postpartum

(dpp) for periods ranging from 12 to 96 h, favored the number of cows displaying overt signs of estrus (Escrivao et al., 2012). In general, longer separations or repeated calf withdrawals result in higher cow pregnancy rates with reduced periods of anestrus postpartum (Williams, 1990; Martins et al., 2012). In addition, recent information suggests that following an estrous synchronization protocol including two calf separation periods, cows may display follicular activity as early as 25 dpp preparing the animals for an eventual pregnancy (Pérez-Torres et al., 2015). During these periods of separation, mothers and young undergo important changes in diet, udder pressure, as well as in social structure involving the rupture of their mother-young attachment. These changes are con-

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sidered stressful events negatively affecting behavior, welfare and probably, productivity of the animals (Blecha et al., 1984). According to Marquezini et al. (2013), the negative impact varies according to the age of the calf and the intensity of human intervention. In spite of the importance of this topic and the large amount of work published (see review from Yavas and Walton, 2000), there is comparatively little information on the wellbeing of calves and dams during these procedures, particularly in Brahman cattle, where it has been demonstrated that a pre-synchronization program at day 25 including calf separation could trigger the onset of ovarian activity and facilitate a breeding program at day 50 after a second separation (Pérez-Torres et al., 2015). The aim of this study was to investigate the effects of separation time on behavioral and physiological characteristics of Brahman cows and their calves with a hypothesis that most of the stress indicators in cows return to basal levels after two days of separation, and that younger calves display less stress signs than older calves.

2. Material and methods

2.1. Ethics

The methods used in the present experiment were approved by the Ethical committee for Experimentation in Animals of the Faculty of Livestock and Agricultural Sciences, University of the State of Morelos Mexico in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2. Location

The study was undertaken at the Centre for Teaching, Research and Extension in Tropical Animal Husbandry belonging to the Faculty of Veterinary Medicine of the National Autonomous University of Mexico, located in the State of Veracruz, Mexico at 20°4' N and 97°3' W. The climate is hot and humid, without a defined dry season. Average yearly rainfall is 1840 mm and average temperature ranges between 14 and 35 °C.

2.3. Animals

Thirty Brahman multiparous cows with three to six parturitions and between five and nine years of age were grazed on *Cynodon nlemfuensis* pasture from June to September 2013. Average rainfall for the experimental period was 674 mm and temperature, 26 °C.

2.4. Treatments

Cows were randomly assigned to one of three temporary weaning duration ($n = 10/\text{group}$): (1) 24 h, (2) 48 h, (3) 72 h, balanced for age of the cows. Treatment was applied at 25 and 45 dpp, with the same cow-calf pairs allocated to the same weaning duration treatment at both ages. Separation treatments were applied starting between 8:00 and 10:00 h. At the end of each temporary weaning period, calves were returned to their dams. Immediately before each separation, calves had identifying ribbons tied around their necks to facilitate observation at a distance. Cows had large numbers painted on their flanks using hair dye (Majirel™, from LíOréal professionnel®, Fabrel S.A. de C.V., Mexico City, Mexico) which is a common procedure in our laboratory. Estrus was synchronized twice, at day 16 and day 36 after calving using a controlled internal drug release device (Easy-breed CIDR™, 1.9 g natural progesterone Zoetis®, Mexico city, Mexico), which remained in situ for nine days. CIDR release was withdrawn at day 25 and 45, respectively. Temporary weaning started at the time of CIDR removal.

A 16% protein commercial concentrate was offered to the calves, and mineral salt and water were available *ad libitum*. Cows

remained at all times on the original four-ha *Cynodon* pasture. Each cow also received 2 kg/day of a commercial concentrate containing 12% protein to meet their maintenance and average lactation requirements. Mothers and their young were separated by a 50 m wide wire-fenced, empty paddock but in earshot and sight of each other during the duration of the experiment. During the separation period, calves remained in a four-ha *Cynodon* pasture, similar to the one where they use to stay with their mothers.

2.5. Measurements

Observations were performed every 20 min from 7:00 to 18:00 h by four observers using instantaneous sampling (Martin and Bateson, 1993); two were assigned to the cows and two to record calf data. The observer minimum distance to the animals was maintained at 10 m.

Behaviors recorded include the proximity of the cows and calves to the fence, and animals vocalizing and/or lying down. The distance between mother and young was determined by measures of diminishing proximity to the fence line separating the two paddocks <10; >10 and <20; and >20 m.

The number of vocalizations uttered was recorded continuously over eleven periods of 10 min every hour during the observation period.

Blood samples were taken for analysis of serum cortisol immediately before the separation period and every 24 h during separation; in cows from the coccygeal vein and from the calves in the jugular vein using vacutainer tubes without anticoagulant. Samples were centrifuged at 3000 rpm for 30 min and 1 ml of serum was stored at –20 °C for subsequent cortisol analysis using commercial coated tube RIA kits (Pantex, Santa Monica, CA) following standard procedures (Farmer and Pierce, 1974).

Blood samples were collected during the routine movement of the herd. All cows and calves were moved individually in a calm manner from a crowd pen to a semicircular solid metal-sided single-line raceway that led to a chute system. There, cows were immobilized and blood samples were collected. Similarly, during the separation period, after the last cow was sampled and the herd was driven to their paddock, calves were gently driven to the single line raceway and blood sample was collected.

Calves were weighed individually at 25 and 45 days of age, immediately before and after separation from their dams to estimate weight changes during this period. By the end of the dam-calf separation, milk consumption was estimated by weighing calves before and after (weigh-suckle-weigh procedure) their first suckling episode on reuniting with their dams.

2.6. Statistical analysis

The unit of analysis was each individual animal. All the variables were tested for normal distribution by the Shapiro–Wilk test. A mixed model analysis of variance (ANOVA) with repeated measures was performed for cortisol level, with duration of separation treatment (24, 48 and 72 h) and the age of the calves (25 or 45 d) and their interactions as fixed effects and the animal within treatments as a random effect. Age and lactation ranks were previously standardized. The proportion of cows and calves at different distances from the fence line was analyzed by comparing the proportions in the two independent groups (Altman, 1991). Body weights before and after separation were compared using a Paired-Sample t-test, while behavioral variables using categorical data from the number of animals lying down, vocalizing and vocalizations per hour, were analyzed by the use of Chi-square tests for $r \times k$ tables (Gill, 1978), where r corresponded to the duration of separation, and k , to the

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