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Overstocking dairy cows during the dry period affects dehydroepiandrosterone and cortisol secretion

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ABSTRACT

Stressful situations trigger several changes such as the secretion of cortisol and dehydroepiandrosterone (DHEA) from the adrenal cortex, in response to ACTH. The aim of this study was to verify whether overstocking during the dry period (from 21 ± 3 d to the expected calving until calving) affects DHEA and cortisol secretion and behavior in Holstein Friesian cows. Twenty-eight cows were randomly divided into 2 groups (14 animals each), balanced for the number of lactations, body condition score, and expected date of calving. Cows in the far-off phase of the dry period (from 60 to 21 d before the expected calving date) were housed together in a bedded pack. Then, animals from 21 ± 3 d before the expected calving until calving were housed in pens with the same size but under different crowding conditions due to the introduction of heifers (interference animals) into the pen. The control condition (CTR) had 2 animals per pen with 12.0 m^2 each, whereas the overstocked condition (OS) had 3 interference animals in the same pen with 4.8 m^2 for each animal. On d −30 ± 3, −21 ± 3, −15 ± 3, −10 ± 3, and −5 ± 3 before and 10, 20, and 30 after calving, blood samples were collected from each cow for the determination of plasma DHEA and cortisol concentrations by RIA. Rumination time (min/d), activity (steps/h), lying time (min/d), and lying bouts (bouts/d) were individually recorded daily. In both groups, DHEA increased before calving and the concentration declined rapidly after parturition. Overstocking significantly increased DHEA concentration compared with the CTR group at d −10 (1.79 ± 0.09 vs. 1.24 ± 0.14 pmol/mL), whereas an increase of cortisol was observed at d −15 (3.64 ± 0.52 vs. 1.64 ± 0.46 ng/mL). The OS group showed significantly higher activity (steps/h) compared with the CTR group. Daily lying bouts tended to be higher for the OS group compared with CTR group in the first week of treatment. The overall results of this study documented that overstocking during the dry period was associated with a short-term changes in DHEA and cortisol but these hormonal modifications did not influence cow behavior.

Key words: dairy cattle, cortisol, dehydroepiandrosterone, overstocking, dry period

INTRODUCTION

Stressful situations trigger several changes such as activation of the sympathetic nervous system and hypothalamic-pituitary-adrenal axis. As a consequence, the adrenal cortex, in response to ACTH, starts to secrete both cortisol and dehydroepiandrosterone (**DHEA**). Cortisol and DHEA are produced in different sections of the adrenal cortex; the zona fasciculata secretes cortisol and the zona reticularis secretes DHEA and its sulfated metabolite dehydroepiandrosterone sulfate (**DHEA-S**; Nguyen and Conley, 2008). In female primates, DHEA and DHEA-S are also produced in the ovary (Sirinathsinghji and Mills, 1983), and in primates and rodents DHEA is produced within the central nervous system and in peripheral nerves (Baulieu, 1998).

Cortisol stimulates the mobilization of the energy needed to overcome stressors; DHEA and DHEA-S are androgen precursors that have been shown to exert antioxidative and anti-inflammatory effects (Kalimi et al., 1994; Maninger et al., 2009) and to play a protective and regenerative role (Maninger et al., 2009; Theorell, 2009).

In humans, an acute psychosocial stress induces a DHEA and DHEA-S increase (Izawa et al., 2008; Lennartsson et al., 2012), whereas long-term psychosocial stress negatively affects both steroids levels (Izawa et al., 2012; Lennartsson et al., 2013). Elevated levels of DHEA and DHEA-S in response to the stressor have been found in both men and women, along with significantly increased ACTH, cortisol, heart rate, and blood pressure. Modifications in DHEA release in response

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to stressors have been observed also in the bovine species. A 23% decrease in serum DHEA and 65% higher cortisol:DHEA ratio were observed in lame cows compared with sound cows (Almeida et al., 2008), and a 1.6-fold DHEA decrease was observed in the plasma of transportation-stressed bulls (Sporer et al., 2008).

In cows, as in most nonprimate mammals, circulating DHEA-S is significantly lower than DHEA, which can be considered an indicator of the P450c17 enzyme activity and the most important circulating precursor of ectopic androgen and estrogen synthesis. Conversely, DHEA-S contribution as an androgen reservoir is rather limited (Feher et al., 1977; Marinelli et al., 2007). In the bovine, DHEA concentrations are quite variable between individuals in both female (Marinelli et al., 2007) and male (Simontacchi et al., 2004) animals.

Increased stocking density is a common practice among dairy producers; the behavioral consequences of this practice are well documented, whereas the physiological ones have still not been thoroughly investigated. Fregonesi et al. (2007a) observed in dairy cows a linear reduction in lying time as freestall stocking density increased, whereas Huzzey et al. (2006) observed a linear reduction in feeding time as stocking density at the feed bunk was increased. Moreover, increased aggressive displacements are often observed at the overstocked feed bunk or freestalls (Huzzey et al., 2006; Fregonesi et al., 2007b); these competitive environments can make it difficult for some cows to gain access to feed. As for the physiological consequences of overstocking, previous works have shown that cows regrouped into a high stocking density group (Friend et al., 1977) or subjected to overcrowding in the resting area (Friend et al., 1979) presented a higher cortisol response to ACTH challenge compared with cows that were not regrouped or overcrowded, respectively.

In contrast to cortisol, DHEA and DHEA-S have received little attention within the stress research area of domestic animals and no studies so far have investigated the effect of overcrowding on DHEA secretion.

Therefore, the aim of this study was to verify whether overstocking during the dry period affects DHEA and cortisol secretion and the behaviors of activity, rumination, resting, and lying time in Holstein Friesian cows.

MATERIALS AND METHODS

Animals, Housing, and Diet

Twenty-eight Holstein dairy cows were enrolled in this experiment. All animals were housed at the farm of the University of Bologna (Ozzano Emilia, Italy) and used according to EEC animal care guidelines. The experimental procedures had been approved by the Ethical Committee of Bologna University.

Animals were randomly divided into 2 groups (14 animals each), balanced for number of lactations (1.35 $\pm$ 1.31), BCS (3.58 $\pm$ 0.35), and expected date of calving. Cows in the far-off phase of the dry period (60 to 21 d before the expected calving date) were housed together in a bedded pack and received water and grass hay ad libitum. From 21 $\pm$ 3 d until calving, animals were housed in 2 bedded-pack groups where they had ad libitum access to water and were fed daily using TMR. After calving, cows were housed together in a bedded pack area for the first 2 wk of lactation and then moved to a free-stall pen in a group composed of 20 cows overall for the rest of lactation. The TMR were fed approximately at 0700 h for lactating cows and 0900 h for dry cows. The TMR samples were collected weekly throughout the study and analyzed for the chemical composition according to the following methods: DM was determined by gravimetrically drying the sample at 103°C to a constant weight, and CP, amylase- and sodium sulfite-treated NDF with ash correction (**aNDFom**), ADF, and ADL were determined according to Mertens (2002), and AOAC 973.18 (AOAC, 1990), respectively. Starch was determined according to AOAC official method (AOAC 996.11) and ether extract according to AOAC 920.390020. Diet composition and analysis for both dry period and lactation are shown in Table 1.

Experimental Design, Blood Sampling, and Hormone Assays

Animals from 21 d before the expected calving until calving were housed in pens with the same size (24.0 m^2 in total with 15.5 m^2 of resting area and 8.5 m^2 of feeding area) but in different crowding conditions due to the introduction of heifers into the pen (interference animals) having a BW of 500 to 550 kg. In particular, the control condition (**CTR**) had 2 animals per pen with 12.0 m^2 each, whereas the overstocked condition (**OS**) had 3 interference animals in the same pen with 4.8 m^2 for each animal. Interference animals were part of the group during the far-off dry period, to avoid social stress at the introduction. Bunk space was 3.3 m long and designed with a neck rail allowing a space of 1.65 m/head for each CTR animal and 0.66 m/head for each OS animal. Resting area was a deep-bedded pack with straw added twice a day.

On d -30 ± 3, -21 ± 3, -15 ± 3, -10 ± 3, -5 ± 3 before and 10, 20, and 30 d after calving, blood samples were collected from each cow from a jugular vein for the determination of plasma DHEA and cortisol concentra-