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Relationship of concentrations of cortisol in hair with health, biomarkers in blood, and reproductive status in dairy cows

Tracy A. Burnett,* Augusto M. L. Madureira,* Bruna F. Silper,* Abdolmansour Tahmasbi,† Audrey Nadalin,‡ Douglas M. Veira,‡ and Ronaldo L. A. Cerri*[1]
*Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC,V6T 1Z4 Canada
†College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran 91775-1163
‡Agriculture & Agri-Food Canada, Agassiz, BC, V0M 1A0 Canada

ABSTRACT

Hair cortisol has been used to measure chronic stress in dairy cows as it offers the advantage of being noninvasive, fast, and able to indicate levels of cortisol over long periods. The aim of this study was to determine the associations between hair cortisol with clinical disorders, reproductive status, and the development of subclinical endometritis in dairy cows. Furthermore, we aimed to determine the association between hair cortisol concentrations and blood markers associated with metabolic status and acute inflammation. In experiment 1, cows (n = 64) were hair sampled every 3 wk from the tail switch beginning at calving (d 0) until d 126 for cortisol analysis; blood samples were collected every 3 wk from d 0 until 42 for β-hydroxybutyrate and glucose analysis. In experiment 2, cows (n = 54) were chosen retrospectively by diagnosis of subclinical endometritis (END), subclinical endometritis and at least 1 clinical disease (END+CLIN), or as healthy (control) using a cytobrush and ultrasonography at 30 ± 3 d in milk. At the same time, animals were hair sampled for cortisol analysis and blood sampled for haptoglobin and ceruloplasmin analysis. Health records were recorded throughout both experimental periods. Animals with clinical disease presented higher cortisol concentrations than clinically healthy animals in experiment 1 [geometric mean (95% confidence interval); 8.8 (7.8, 9.9) vs. 10.7 (9.6, 12.0) pg/mg]; however, animals diagnosed with subclinical endometritis in experiment 2 did not differ in hair cortisol concentrations [11.7 (9.8, 14.0), 12.2 (9.3, 15.9), 10.5 (8.1, 13.6) pg/mg for control, END, and END+CLIN, respectively]. In experiment 1, an effect of sample day was noted, where d 21 had higher cortisol concentrations than d 42, 84, and 126, but not from d 0 for both parities. Within both experiments, a parity effect was present where multiparous animals consistently had higher cortisol concentrations than primiparous animals. Multiparous cows that became pregnant by 100 d postpartum had lower concentrations of hair cortisol at d 42 and 84 in milk. Lastly, other biomarkers associated with metabolic status and acute inflammation, such as glucose, β-hydroxybutyrate, haptoglobin, and ceruloplasmin, were not strongly correlated with measurements of cortisol in hair. Overall, hair cortisol measurements appear to be associated with clinical disorders and have a direct association with pregnancy status; however, concentrations of hair cortisol may not be suited to differentiate situations of stress with lower magnitudes, such as the development of subclinical disease.

Key words: chronic stress, disease, hair cortisol, dairy cattle

INTRODUCTION

Chronic stress, characterized as a sustained stress response or the excessive secretion of stress mediators, has been shown to impair the immune response and reproductive function in animals (Matteri et al., 2000; Chrousos, 2009). In cattle, several reports have shown greater concentrations of cortisol in plasma in different scenarios involving clinical diseases and inflammatory responses. Comin et al. (2013) reported greater concentrations of cortisol in hair from clinically compromised (e.g., laminitis, metritis, or mastitis) cows compared with those with an absence of disease. Additionally, Lavon et al. (2008) observed that animals administered *Escherichia coli* endotoxin showed an increase in cortisol in plasma, suggesting that an inflammatory response induces increases in cortisol. Some researchers have reported that an inflammatory infection caused by *E. coli* endotoxins administered into the uterine lumen has negative effects on reproduction, which suggests this may be due to the release of cortisol as a result of the inflammation (Lopez-Diaz and Bosu, 1992; Huszenicza et al., 2004). However, it is unclear if subclinical states of common disorders in dairy cows,

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[1]Corresponding author: ronaldo.cerri@ubc.ca

such as subclinical endometritis and mastitis, trigger the same corticotropin-releasing hormone responses from the hypothalamus or if these responses can be measured using hair samples. In addition, increased concentrations of cortisol are known to cause immunosuppressive effects in dairy cattle and swine (Hopster et al., 1998; Tuchscherer et al., 2004) and are linked to decreases in antibody concentrations and reductions in phagocytic functions in monocytes and macrophages (Roth, 1985).

Chronic and acute stress can trigger disruptions in reproductive function. Chronic lameness in cattle, for example, is linked to disruptions in the pulsatile pattern of GnRH and consequent impairment of estrous behavior expression (Walker et al., 2008). Acute stress, characterized as short-term and normally measured through the blood, saliva, and feces, has detrimental effects on ovarian cyclicity by disrupting the normal release of hormones from the hypothalamic-pituitary-gonadal axis that controls reproduction (Dobson et al., 2003; Smith et al., 2003). Stressors, such as isolation, transport, and restraint (acute causes), have been found to interfere with the endocrine events preceding ovulation and, thus, result in ovulation failure (Moberg, 2000).

Most of the literature on dairy cattle concerning cortisol measurements has been done in plasma (Forslund et al., 2010), saliva (Negrão et al., 2004), or fecal samples (Huzzey et al., 2011). Alternatively, the measurement of cortisol in hair has recently received attention and has the advantage of being a noninvasive procedure (Comin et al., 2013; Burnett et al., 2014); however, no research has demonstrated the dynamic changes on how disease events (clinical and subclinical) and reproductive status are associated with concentrations of cortisol in hair over time. In addition, the relationship between cortisol in hair, energy metabolites, and acute-phase proteins is currently unclear and can provide valuable information about the validity of hair cortisol tests.

The aim of our study was to determine the associations between hair cortisol with clinical disorders, reproductive status, and the development of subclinical endometritis in dairy cows. Furthermore, we aimed to determine the association between cortisol in hair with energy metabolites in blood (glucose and BHBA) and acute-phase proteins in plasma (ceruloplasmin and haptoglobin). It was hypothesized that (a) the concentration of cortisol found in the hair of dairy cows has a negative relationship with the health and reproductive status of dairy cows, and (b) a positive association exists between concentrations of cortisol in hair and concentrations of BHBA, glucose, ceruloplasmin, and haptoglobin in blood during the transition period.

MATERIALS AND METHODS

This experiment was conducted between June 2011 and March 2012 at the University of British Columbia's Dairy Education and Research Centre (Agassiz, BC, Canada). All procedures were approved by the Animal Care Committee of the University of British Columbia. Animals used in this experiment were cared for as outlined by the guidelines provided by the Canadian Council for Animal Care (2009).

Animals and Housing

A total of 118 high-producing Holstein dairy cows were used in this study, which consisted of 2 experiments (experiment 1: n = 64; experiment 2: n = 54). Cows produced (mean ± SD) 12,236 ± 2,219 kg of milk (305-d mature-equivalent yield) and with BCS ranging from 1.75 to 3.25 at 30 ± 3 DIM. All animals were housed in the same naturally ventilated wooden-framed barn with a freestall design, equipped with deep sand-bedded stalls. Animals were milked twice daily at 0500 and 1500 h with automatic milking machines. Fresh TMR was delivered twice daily at approximately 0700 and 1600 h. The TMR was formulated following the NRC guidelines (NRC, 2001) to meet or exceed the requirements of a 620-kg Holstein cow producing 40 kg/d of 3.5% FCM; the animals had ad libitum access to both TMR and water.

During both experimental periods, various potential forms of stressors were recorded, including retained placenta, clinical hypocalcaemia, displaced abomasum, bouts of clinical mastitis and metritis, surgical procedures, milk yield (305-d mature-equivalent yield), and SCC. All animals were body condition scored at 30 ± 3 DIM on a 5-point scale from thin (1) to obese (5) as outlined by Edmonson et al. (1989). Animals were later classified as thin (BCS <2.75), average (BCS = 2.75), or moderate (BCS >2.75). All health and production information was collected by the dairy herd personnel with the assistance of the herd veterinarian, and confirmed and recorded by the project leader using the on-farm Dairy Comp 305 software (Valley Agricultural Software, Tulare, CA). Animals were considered healthy if they had an absence of clinical disease during the entire experimental period, or as clinically diseased if they were diagnosed with 1 or more clinical disease event.

Clinical mastitis was diagnosed by inspection for udder hardness, heat, and clots or discolored milk and with the use of the California Mastitis Test. Retained placenta was defined as the failure to expel the placenta within 24 h postpartum (LeBlanc, 2008). Clinical