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Short communication: Is hair cortisol a potential indicator for stress caused by chronic lameness in dairy cows?

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ABSTRACT

The objective of this study was to evaluate hair cortisol concentration as an indicator for stress caused by chronic lameness in dairy cows. Sixty-eight cows were scored for lameness for 4 consecutive weeks. The hair of the tail switch was clipped at the beginning of the study and regrown hair was clipped after 4 wk. Hair samples were analyzed for cortisol concentration. Animals with 2 consecutive locomotion scores $\geq$3 or with an overall mean score >1.5 were classified as lame. After pair matching lame and nonlame cows, considering days in milk, lactation number, and milk yield, and excluding cows with less than 20 mg hair sample for analysis, 21 lame and 21 nonlame cows were included in the analysis. The mean hair cortisol concentration in this study was 2.32 $\pm$ 0.35 pg/mg (mean $\pm$ standard deviation). Cortisol concentration from hair regrown in the study period was 2.38 $\pm$ 0.95 and 2.26 $\pm$ 1.35 pg/mg for lame and nonlame cows (n = 21), respectively; we found no difference in mean cortisol level of primiparous and multiparous cows. Based on these data, hair cortisol concentration was not a useful indicator to differentiate cows with chronic lameness and healthy cows.
Key words: dairy cow, hair cortisol, lameness, pain

Short Communication

Welfare of food-producing animals is a growing of concern of consumer. Producers and retailers are in need of reliable and easy-to-assess rating systems to evaluate and label the welfare standard of food of animal origin. For farm animals, Oliver et al., (2004) developed 5 freedoms as objectives for animal welfare: freedom from hunger and thirst, fear, discomfort, to express normal behavior, and freedom from pain. Although, measuring pain in animals is challenging, estimating stress as a result of pain or discomfort is an established method (Anil et al., 2002); therefore, the assessment of stress in farm animal would be a valuable tool for evaluating animal welfare.

Determination of hypothalamus-pituitary-adrenal axis activity is the standard procedure to evaluate stressful conditions in farm animals (Mormède et al., 2007). Cortisol can be analyzed from different sources, such as blood, saliva, feces, and hair. Either acute or chronic stress could be quantified through measurement of changes in physiological parameters, such as heart rate, heart rate variability, blood pressure, and levels of various metabolic hormones. Hair cortisol concentration can be a valuable biomarker of chronic stress. The fairly constant growth rate of hair enables the investigator retrospectively to examine cortisol production for a given period of time (Russell et al., 2012). However, it is still elusive to interpret the extent to which changes in circulating levels of cortisol can reflect the acute, chronic, or diurnal variations (Do Yup Lee and Choi, 2015). Handling and restraining of dairy cattle, however, has been shown to rapidly increase concentrations of cortisol in plasma, leading to confounding results (Cook et al., 2000). Sampling concerns have contributed to the need for noninvasive cortisol sampling methods that can reflect long-term increases in cortisol (Moberg and Mench, 2000). In dogs and rhesus macaques, it has been shown that hair cortisol concentration is correlated with concentration of cortisol in saliva (Bennett and Hayssen, 2010; Davenport et al., 2006). In humans, hair cortisol concentrations have been found to have a positive correlation with chronic stress (Thomson et al., 2010; Pereg et al., 2011). The examination of hair to evaluate long-term circulating cortisol levels could be an effective and noninvasive tool to provide an indication of the overall noxiousness of the experience, which includes both physically and emotionally stressful components (Moya et al., 2013). Measuring cortisol in hair is considered to be a valid method to monitor the exposure of an animal to situations that will increase cortisol levels over time, as occurs in chronic stress (Comin et al., 2013).

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Most recently, a study conducted with dairy cows suggested that the tail switch is a good location for hair sampling from black and white Holsteins for the analysis of cortisol due to the consistent white-colored hair, greater growth rates, and easy access (Burnett et al., 2014). Furthermore, the measurement of cortisol concentrations in hair could detect significant differences comparing healthy with clinically diseased (i.e., retained placenta, clinical hypocalcemia, displaced abomasum, clinical mastitis, metritis, and surgical procedures) lactating cows (Burnett et al., 2015).

Lameness is perhaps the biggest challenge for dairies to overcome (Bicalho et al., 2007). The cow-level prevalence of clinical lameness ranges from 26 to 54% (Bicalho et al., 2007), but it seems to be extremely underestimated by herd managers (Espejo et al., 2006). Lameness can reproducibly be assed (Sprecher et al., 1997) and has been classified as the most representative animal-based welfare indicator in dairy cattle (Whay et al., 2003). Foot lesions causing lameness in cattle are multifactorial, contributing factors being trauma, metabolic disorders, and infection (Walker et al., 2008; Leach et al., 2010). Pain caused by lameness could act as a stressor in dairy cattle (Underwood, 2002). Adverse situations trigger responses of the adrenals, which result in an increase in glucocorticoid (Möstl and Palme, 2002). Cortisol has been employed as a stress biomarker in lame cattle (Bustamante et al., 2015). On the day of diagnosis, serum cortisol concentrations are elevated in cows diagnosed with lameness (O'Driscoll et al., 2017).

Therefore, the objective of our study was to compare concentrations of hair cortisol between healthy and chronically lame cows. We wanted to test the hypothesis that chronic lameness leads to long-term stress that can be identified and assessed via hair cortisol concentrations.

All procedures were approved by the Animal Care Committee of Brandenburg, Germany (AKZ: 2340–8-2016). The study was conducted on a commercial dairy farm in Brandenburg, Germany, between September and October 2016. The farm was milking 430 cows and herd-average 305-d milk yield was 9,475 kg (4.0% fat and 3.5% protein). Cows were housed in a freestall barn on deep-bedded straw and concrete flooring. They were fed a TMR with corn (66%) and grass (34%) silage. Animals were milked twice daily at 0400 and 1500 h in a herringbone parlor. The farm was visited on a weekly basis and all cows between 60 and 150 DIM were enrolled. After excluding cows that developed a clinical disease other than lameness (i.e., endometritis, ketosis, mastitis, displaced abomasum) during the investigation period diagnosed by the local veterinarian and cows that could not be scored for lameness for 4 consecutive weeks, 68 cows were included in the study. Hoof trimming was not conducted nor were analgesic drugs administered to cows enrolled in the study. Animals were considered healthy if they had no signs of clinical disease during the entire experimental period. Cows were 47.3 ± 15.2 (mean ± SD) months old, had an average of 2.28 ± 1.23 (mean ± SD) lactations, and an average milk yield on day of inclusion of 33.1 ± 6.2 kg (mean ± SD) of milk.

On the day of enrolment a general clinical examination was performed. Lameness was scored using a 5-point scale ranging from 1 to 5 [1 = normal, 2 = presence of a slightly asymmetric gait, 3 = the cow clearly favored 1 or more limbs (moderately lame), 4 = severely lame, 5 = extremely lame (nonweight-bearing lame)] according to Sprecher et al. (1997). Visual locomotion scoring was conducted once weekly for 4 consecutive weeks by the same observer. At the day of enrollment the hair at the tail switch was clipped in all cows with surgical scissors. The tip of the tail was then stained with marking spray (Raidex, Dettingen, Germany). After 4 wk (i.e., last day of scoring), the regrown hair at the tail switch was cut again. First, all segments colored from marking spray were removed, and then hair was cut as close as possible to the tip of the tail. Regrown hair length varied between 0.8 and 1.2 cm. According to Burnett et al. (2014), only the white hairs of this sample were collected and stored at room temperature in dark plastic bags and sent to the laboratory (Technical University, Dresden, Germany, EU accreditation number: D-PL-14016–01–00) for investigation. Hair samples were washed 3 times in a 20-mL glass scintillation vial (NeoLab, Heidelberg, Germany), adding 2.5 mL isopropanol for 3 min. Hair samples were grinded with a Retsch ball mill (MM 400, Haan, Germany) for 5 min at 30 Hz. Twenty milligrams of the sample were weighed out and transferred into a 3-mL glass scintillation vial (IBL, Hamburg, Germany). For steroid extraction, 1.8 mL of HPLC-grade methanol (Roth, Karlsruhe, Germany) were added and these vials then slowly rotated (Heidolph Titramax 1000, Schwabach, Germany) over 18 h. Samples were centrifuged at 2,000 × g at room temperature for 2 min, and 1.6 mL of the clear supernatant was transferred into a new glass vial. Alcohol was evaporated at 50 °C under a constant stream of nitrogen until the samples were completely dried. The residues were resuspended with 225 μL of double-distilled water and vortexed for 15 s. For analysis, 100 μL were tested with a commercially available immunoassay for salivary cortisol with luminescence detection (LIA, IBL-International, Hamburg, Germany). All hair samples were processed in duplicates. The inter- and intra-assay coefficients of variation for hair cortisol were 12.7 and 9.8%, respectively.