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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 enrollment 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.

Animals with 2 consecutive scores ≥3 or with an overall mean score >1.5 were classified as lame (Morris et al., 2011). For the analysis, the lame cows were pair-matched to a nonlame cow considering DIM, parity, and average milk yield in the study period. Thirty-two cows were classified as healthy (mean score = 7.04, SD = 1.34) and 36 were classified as lame [Ø (average) score = 14.84, SD = 2.80]. Twelve and 14 cows had to be excluded from analysis because hair sample size was below 20 mg, the minimum for the cortisol assay and a matching cow was not available on a given day. After pair-matching every lame cow to a nonlame cow considering number of lactation and average milk yield, 42 cows, 12 primiparous and 30 multiparous, remained for final analysis (Table 1). Data were entered into Excel spreadsheets (version 2013; Microsoft Corp., Redmond, WA) and analyzed using SPSS for Windows (version 22.0; SPSS Inc., Munich, Germany).

Normality of distributions of continuous parameters (cortisol concentration) was assessed by plotting and visually examining the data, calculating a Q-Q plot and using the Kolmogorov-Smirnov test. Equality of variances was tested using Levene's test. Difference in cortisol concentration between primiparous and multiparous were tested with t-test. Generalized linear mixed models were used to determine the effect of lameness, parity, and average milk yield on the concentration of cortisol in cows. Average milk yield was considered as a random effect. Interactions were tested for all relevant parameters.

The mean hair cortisol concentration in this study was 2.32 ± 0.35 pg/mg (mean ± SD) for all cows (n = 42). This is in the same range as described in previous studies [Moya et al. (2013): 2.35 ± 1.76 pg/mg; Comin et al. (2013); 2.5 ± 0.1 pg/mg], but lower than data reported by González-de-la-Vara et al. (2011; 12.15 ± 1.85 pg/mg) or Burnett et al. (2015; 9.8 ± 3.7 pg/mg). Hair cortisol concentration within the same species can be effected by environmental factors, such as climate (Ghassemi Nejad et al., 2017) and nutrition (Moya et al., 2015), or cow-level factors, such as hair color (Burnett et al., 2014) or breed (Peric et al., 2013). The high variability of results in different studies indicate that it may be difficult to find a threshold for hair cortisol concentration indicative of stress in dairy cows kept in different environments. Furthermore, the method used may have an influence on variability. As in the current study, immunoassays are commonly used to measure saliva, blood, urine, and hair cortisol concentrations. These methods, although sensitive to changes, are presently subject to interassay variability, precluding a unified definition of physiologic ranges of levels (Russell et al., 2012). In our study the interassay variability was 12.6%, which was higher compared with 7.4% in the study of Burnett et al. (2014) or 9.6% in the study of Comin et al. (2013).

Table 1. Cortisol concentration (pg/mg) of hair samples collected after 4 wk of lameness in tail switch region of nonlame (n = 21) and lame (n = 21) dairy cows

Cortisol (pg/mg)	Parity	No. of cows	Mean	SD
Nonlame	All	21	2.384	0.957
	Primiparous	6	2.143	0.582
	Multiparous	15	2.480	1.074
Lame	All	21	2.263	1.686
	Primiparous	6	2.743	2.008
	Multiparous	15	2.072	1.576

It seems to be difficult to define a baseline cortisol concertation for a not stressed individual or group of animal. For evaluation of the effect of a stressor, a comparison of cortisol concentration with or without stressor would be necessary (Bertulat et al., 2013). In the present study, lameness was observed in only 1 farm over a 4-wk study period and was assumed as the cause of chronic stress and was compared with healthy cows kept in the same pen. Cortisol concentration from hair regrown in the study period was 2.38 ± 0.95 and 2.26 ± 1.35 pg/mg for lame and nonlame cows (n = 21), respectively ($P = 0.27$; Figure 1). In the group of lame cows, 13 were observed with a severe lameness score ≥4 at least once in the 4-wk observation period. Cortisol concentration in this subgroup was 2.24 ± 1.2 pg/mg ($P = 0.4$). We also found no difference in mean cortisol levels of primiparous and multiparous cows ($P = 0.73$). Lameness ($P = 0.27$), lameness score ($P = 0.24$), parity ($P = 0.34$), and average milk yield ($P = 0.38$) had no effect on concentration of hair cortisol. This is in contrast to a previous study (Comin et al., 2013) in which hair cortisol concentration of clinically (i.e., laminitis, metritis, mastitis) and physiologically compromised (parturition) cows was higher (5.12 pg/mg; n = 218) than those of clinically healthy cows (3.29 pg/mg; n = 257). In Comin et al. (2013), data from the on-farm computer were used to group the animals; thus, it is likely that the personnel-based diagnoses included more acute and severe lame cows. The study of O'Driscoll et al. (2015) demonstrated that, on the day of diagnosis, the cortisol concentration in serum was elevated in cows with sole ulcers. Our data did not show any influence off the lameness score on the hair cortisol concentration, which is in agreement with O'Driscoll et al. (2017), who reported the cortisol concentration in cows with sole hemorrhages. The transition from acute to chronic pain causes an adaptive coping response of the organism and permits return to normal concentrations of cortisol (Hannibal and Bishop, 2014). We could not demonstrate an increase of hair cortisol concentra-

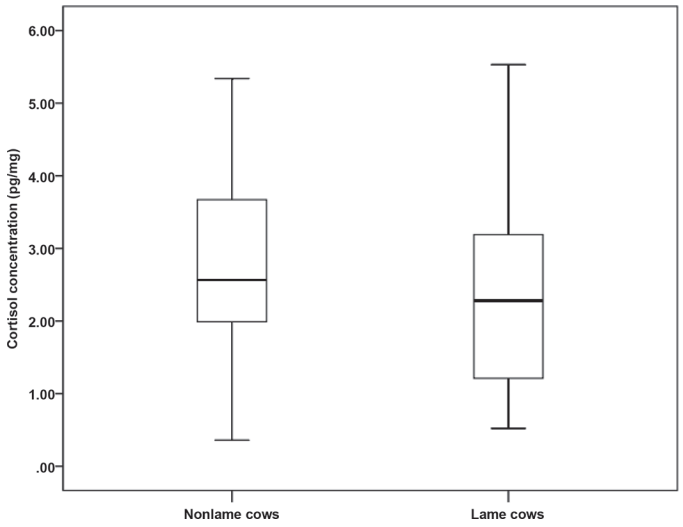

Figure 1. Cortisol concentration (pg/mg) of hair samples collected after 4 wk of lameness in tail switch region of nonlame (n = 21) and lame (n = 21) dairy cows. The central box represents the interquartile range from the first to third quartile. A segment inside the box shows the highest and lowest value within 1.5 times the interquartile range, respectively.

tion in chronically lame cows. Redbo (1998) reported that chronic stress leads to a decrease in sensitivity of the adrenal cortex. Growing bulls responded to a long stress period due to tethering with a lower plasma cortisol concentration after ACTH stimulation compared with a control group (Ladewig and Smidt, 1989).

Hair cortisol concentrations have been shown to represent adrenocortical activity after ACTH challenge during the 14-d interval before hair collection (González-de-la-Vara et al., 2011), indicating its usefulness as a biomarker for acute and painful events in this period for animals kept in the same environment (Comin et al., 2013, Burnett et al., 2015). Reduced secretion of ACTH and cortisol during continued exposure to a stressor, such as chronic lameness, can reflect a mechanism to prevent prolonged exposure to elevated concentrations of cortisol as described by (Knights and Smith, 2007). We could not demonstrate hair cortisol concentration to be valuable biomarker for chronic lameness in dairy cows.

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