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Hair Cortisol Levels to Monitor Hypothalamic-Pituitary-Adrenal Axis Activity in Healthy Dairy Cows

[1]A. Comin, [1]T. Peric, [1]M. Montillo, [2]M. Faustini, [1]V. Zufferli, [3]A. Cappa,
[4]G. Cornacchia and [1]A. Prandi
[1]Department of Food Sciences, University degli Studi di Udine,
via Sondrio 2/a, 33100 Udine, Italy
[2]Department of Veterinary Sciences and Technologies for Food Safety,
University degli Studi di Milano, via Celoria 10,20133 Milano, Italy
[3]Evoluzione S.r.l., via S. Sepolcro 29, 36040 Sossano (VI), Italy
[4]Via Fosso Chiozzo 1/2 Villa Garibaldi, 46037 Roncoferraro (MN), Italy

Abstract: The objective of this study was to evaluate hair cortisol levels in healthy friesian dairy cows. The trial was conducted on 229 multiparous lactating Italian Friesian dairy cows from 90-305 days of lactation selected from a single herd. The animals were clinically healthy and had not undergone social group changes or any disease occurred within the past 3 months. Hair samples were obtained by clippers from the animal's forehead and hair cortisol concentration was evaluated by RIA. The hair cortisol concentrations ranged from 0.8-20.41 pg mg^{-1}. In 50% of the animals, cortisol levels were below 3.13 pg mg^{-1}. The cows were classified into fourteen classes according to their hair cortisol levels and percentage frequencies for each class were calculated. The 33% of the animals were concentrated in the hair cortisol class 2-2.99 pg mg^{-1}. This preliminary study describe the distribution of hair cortisol levels in healthy Friesian dairy cows and suggest hair cortisol analysis as an interesting and useful tool to monitor HPA axis activity in healthy lactating dairy cows.

Key words: Dairy cows, cortisol, hair, health, animals, hormone

INTRODUCTION

Cortisol is a key hormone in the physiological response of the Hypothalamic-Pituitary-Adrenal Axis (HPA) and can be determined in blood or non-invasively, in faeces (Szeto *et al*., 2004) urine (Walker *et al*., 2009) milk (Gygax *et al*., 2006) and saliva (Negrao *et al*., 2004). HPA activity can also be measured by cortisol levels in hair samples. Cortisol plasma spread from capillaries to cells of the hair follicles and may finally be deposited in the hair shaft (Cone, 1996). Hair cortisol levels are unaffected by circadian hormone variations or by factors inducing short-term variations. This property of hair has been exploited to determine chronic exposure to xenobiotic substances (Raul *et al*., 2004; Kintz *et al*., 2006; Cirimele *et al*., 2008). The collection of this biological material is simple, non-invasive and the sample obtained does not decompose like other body fluids or tissues (Balikova, 2005). Measurement of hair cortisol has been validated in humans (Villain *et al*., 2004; Sauve *et al*., 2007), rock hyraxes (Koren *et al*., 2002) non human primates (Davenport *et al*., 2006; Fairbanks *et al*., 2011; Fourie and Bernstein, 2011; Laudenslager *et al*., 2011), dogs and cats (Accorsi *et al*., 2008; Bennett and Hayssen, 2010), grizzly and polar bears (Macbeth *et al*., 2010; Bechshoft *et al*., 2011) and alaskan caribous (Ashley *et al*., 2011). In the dairy cow, hair oestradiol and testosterone levels have been determined (Gleixner and Meyer, 1997) and two prior studies has addressed the use of hair cortisol determinations in bovine (Comin *et al*., 2011; Del Rosario Gonzalez-de-la-Vara *et al*., 2011). The aim of the current study was to evaluate hair cortisol levels in healthy Friesian dairy cows.

MATERIALS AND METHODS

Although, hair sampling is a non invasive and troublesome procedure, the study was carried out in accordance with EU Directive 2010/63/EU for animal experiments.

Animals: The trial was conducted on 229 multiparous lactating Italian Friesian dairy cows from 90-305 days of lactation. The cows were from a single herd and a farm in

Corresponding Author: Tanja Peric, Department of Food Sciences, University degli Studi di Udine, via Sondrio 2/a, 33100 Udine, Italy

which a computerised system was used to store animal production management and health data. This database provided us with clinical and management data for every animal prior to the trial. The animals were subjected to periodic clinical examinations and those that were not in good health were withdrawn from the study. No animal enrolled had experienced a change in social group or any disease occurred in the 3 months before the start of hair sampling.

Hair sampling: Using clippers, hair samples were carefully obtained from the animal's forehead which is the area that is most readily accessible under conditions of intensive management. Samples were collected during routine daily farm activities to avoid stress and in compliance with current legislation on animal welfare. All samples were obtained in May and stored in dry tubes at room temperature until analysis.

Hormone analysis: Hair strands were washed in 5 mL isopropanol (Carlo Erba Reagenti, Italy) as suggested by Davenport *et al.* (2006) to minimize the risk of extracting cortisol from outside the hair and at the same time to ensure the removal of any steroids on the surface of the hair due to sweat and sebum. Hair cortisol was extracted as described by Koren *et al.* (2002) and modified by Accorsi *et al.* (2008) and determined using the RIA Method described by Tamanini *et al.* (1983). The rabbit anti-cortisol antibody used was obtained from biogenesis (Poole, UK). Cross-reactivities of this antibody with other steroids are: cortisol 100%, corticosterone 1.8% and aldosterone <0.02%. Within and between assay coefficients of variation for the method are 5.4 and 9.6%, respectively. The sensitivity of the method is 0.21 pg mg^{-1}. Parallelism was demonstrated between serially diluted hair extracts and the standard curve was 0.99.

Statistical analysis: The variable hair cortisol concentration was submitted to univariate statistical analysis to establish its distribution, central tendency and dispersion. On samples, mean, standard error, median, sample variance, Kurtosis and Skewness were calculated. The Kolmogorov-Smirnov test was used to confirm or refute the normal distribution of data. Fourteen classes of cows according to their hair cortisol concentrations were defined and percentage frequencies for each class calculated.

RESULTS AND DISCUSSION

The hair cortisol concentrations recorded for the 229 dairy cows ranged from 0.8-20.41 pg mg^{-1}, mean and standard error were respectively, 4.02 and 0.17 pg mg^{-1}.

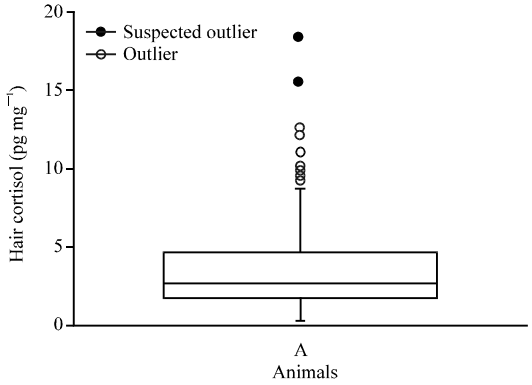

Fig. 1: Hair cortisol levels measured in 229 multiparous lactating Italian Friesian dairy cows. Plots represent the median (horizontal lines), 25th and 75th quartiles (boxes) and the minimum and maximum cortisol values (whiskers)

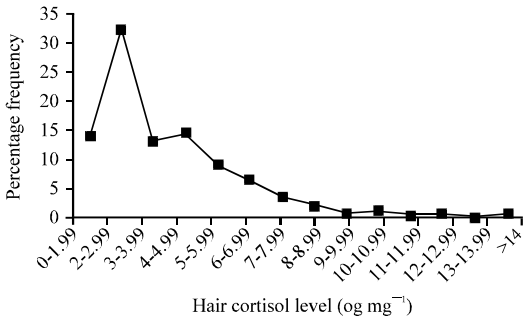

Fig. 2: Percentage frequency curve according to hair cortisol classes

The median, standard deviation and variance were 3.13, 2.62 and 6.89 pg mg^{-1}, respectively. In 50% of the animals, concentrations were <3.13 pg mg^{-1} (median, Q2). This asymmetric distribution along with the values of kurtosis (9.76) and skewness (2.48) obtained indicate that data redistribute according to a non-normal distribution. Hair cortisol levels recorded are shown in the box plot (Fig. 1). The cows were classified into fourteen classes according to their hair concentrations and percentage frequencies for each class were calculated (Fig. 2). The data concentrated in the hair cortisol class 2-2.99 pg mg^{-1}.

This study describe hair cortisol distribution in healthy friesian dairy cows from a single herd. Hair analysis can monitor hormone changes over a long period of time (long-term exposure) and provides a window to the past. This characteristic has been exploited in several studies, both in forensics (Wada *et al.*, 2010; Barroso *et al.*, 2011) and in studies designed to evaluate the response of an animal to adverse environmental conditions (Van Uum *et al.*, 2008; Gow *et al.*, 2010). Assuming a 0.6-1 cm per month hair growth rate in the

dairy cow (Schwertl *et al*., 2003), hair analysis may document a historical timeline of hormonal status from the initial development of a strand of hair to the time of its collection. Accordingly, hair cortisol can be related to a time interval comparable to that estimated for its growth (anagen phase).

In this study, hair cortisol levels were found to vary widely indicating different individual activation of the HPA axis. The most interesting finding was that in a hormonal range from 0.8-20.41 pg mg^{-1}, the 50% of the animals had hair cortisol levels <3.13 pg mg^{-1} and that the class 2-2.99 pg mg^{-1} included 33% of the animals. Considering, as mentioned earlier, that hair cortisol concentrations provide long term information, these levels are likely to be representative of the physiological range of variation in hair cortisol produced in healthy lactating dairy cows.

Interesting results but that require further investigations, regard the presence in the clinically healthy cows both animals with strongly activation HPA axis and animals with a low level of activity.

CONCLUSION

This preliminary study describe the distribution of hair cortisol levels in healthy friesian dairy cows. Further studies are needed to investigate aspects such as the effects of season, temperature and photoperiod on cortisol levels in hair. The analysis of hair cortisol can be an interesting and useful tool to monitor HPA axis activity also in lactating dairy cows. Evaluate the activity of this axis is very important because it regulates many biological processes such as energy balance, reproduction or immune responses (Minton, 1994).

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